

(19)



Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11)

**EP 0 952 221 A2**

(12)

**EUROPEAN PATENT APPLICATION**

(43) Date of publication:  
**27.10.1999 Bulletin 1999/43**

(21) Application number: **99105508.8**

(22) Date of filing: **17.03.1999**

(51) Int Cl<sup>6</sup> **C12N 15/53, C12N 15/60,  
C12N 1/21, C12N 1/20,  
C12P 13/14  
// (C12N1/20, C12R1:01, 1:425)**

(84) Designated Contracting States:  
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU  
MC NL PT SE**  
Designated Extension States:  
**AL LT LV MK RO SI**

(30) Priority **18.03.1998 JP 6906898  
19.10.1998 JP 29712998**

(71) Applicant: **Ajinomoto Co., Ltd.  
Tokyo (JP)**

(72) Inventors:  
• **Izui, Hiroshi c/o Ajinomoto Co., Inc.  
Kawasaki-ku, Kawasaki-shi, Kanagawa-ken (JP)**

- **Ono, Eiji c/o Ajinomoto Co., Inc.  
Kawasaki-ku, Kawasaki-shi, Kanagawa-ken (JP)**
- **Matsui, Kazuhiko c/o Ajinomoto Co., Inc.  
Kawasaki-ku, Kawasaki-shi, Kanagawa-ken (JP)**
- **Moriya, Mika c/o Ajinomoto Co., Inc.  
Kawasaki-ku, Kawasaki-shi, Kanagawa-ken (JP)**
- **Ito, Hisao c/o Ajinomoto Co., Inc.  
Kawasaki-ku, Kawasaki-shi, Kanagawa-ken (JP)**
- **Hara, Yoshihiko c/o Ajinomoto Co., Inc.  
Kawasaki-ku, Kawasaki-shi, Kanagawa-ken (JP)**

(74) Representative: **Strehl Schübel-Hopf & Partner  
Maximilianstrasse 54  
80538 München (DE)**

(54) **L-glutamic acid-producing bacterium and method for producing L-glutamic acid**

(57) L-Glutamic acid is produced by culturing in a liquid culture medium a microorganism belonging to the genus *Enterobacter* or *Serratia* and having an ability to produce L-glutamic acid, which increases in an activity of enzyme catalyzing a reaction for L-glutamic acid bio-

synthesis, or which decreases in or is deficient in an activity of an enzyme catalyzing a reaction branching from a pathway for L-glutamic acid biosynthesis and producing a compound other than L-glutamic acid, and collecting produced L-glutamic acid from the culture medium.

**EP 0 952 221 A2**

## Description

BACKGROUND OF THE INVENTION

[0001] The present invention relates to a novel L-glutamic acid-producing bacterium and a method for producing L-glutamic acid by fermentation using the same. L-Glutamic acid is an important amino acid as food, drugs and the like.

[0002] L-Glutamic acid has conventionally been produced by fermentation methods utilizing the so-called coryneform L-glutamic acid-producing bacteria which principally belong to the genera *Brevibacterium*, *Corynebacterium*, and *Microbacterium* or variants thereof ("Amino Acid Fermentation", Gakkai Shuppan Center, pp. 195-215, 1986). As methods for producing L-glutamic acid by fermentation utilizing other bacterial strains, there have been known the methods utilizing microorganisms of the genera *Bacillus*, *Streptomyces*, *Penicillium* and the like (United States Patent No. 3,220,929), the methods utilizing microorganisms of the genera *Pseudomonas*, *Arthrobacter*, *Serratia*, *Candida* and the like (United States Patent No. 3,563,857), the methods utilizing microorganisms of the genera *Bacillus*, *Pseudomonas*, *Serratia* and the like or *Aerobacter aerogenes* (currently referred to as *Enterobacter aerogenes*) (Japanese Patent Publication (KOKOKU) No. 32-9393(1957)), the method utilizing variant strains of *Escherichia coli* (Japanese Patent Application Laid-Open (KOKAI) No. 5-244970(1993); and the like.

[0003] Though the productivity of L-glutamic acid has considerably been improved by breeding of such microorganisms as mentioned above or improvements of production methods, it is still desired to develop a more inexpensive and more efficient method for producing L-glutamic acid in order to meet the expected markedly increasing future demand of the amino acid.

SUMMARY OF THE INVENTION

[0004] The object of the present invention is to find a novel L-glutamic acid-producing bacterium having a high ability to produce L-glutamic acid, thereby developing a more inexpensive and more efficient method for producing L-glutamic acid.

[0005] To achieve the aforementioned object, the present inventors intensively searched for and studied microorganisms having the ability to produce L-glutamic acid that are different from the previously reported microorganisms. As a result, they found that certain strains derived from microorganisms belonging to the genus *Enterobacter* or *Serratia* had a high ability to produce L-glutamic acid, and have completed the present invention.

[0006] Thus, the present invention provides

(1) a microorganism belonging to the genus *Enterobacter* or *Serratia* and having an ability to produce L-glutamic acid and at least one of the following properties:

(a) the microorganism increases in an activity of an enzyme catalyzing a reaction for L-glutamic acid biosynthesis; and

(b) the microorganism decreases in or is deficient in an activity of an enzyme catalyzing a reaction branching from a pathway for L-glutamic acid biosynthesis and producing a compound other than L-glutamic acid.

(2) a microorganism of the above (1) wherein the enzyme catalyzing the reaction for the L-glutamic acid biosynthesis is at least one selected from the group consisting of citrate synthase (abbreviated as "CS" hereinafter), phosphoenolpyruvate carboxylase (abbreviated as "PEPC" hereinafter), and glutamate dehydrogenase (abbreviated as "GDH" hereinafter).

(3) a microorganism of the above (2) wherein the enzyme catalyzing the reaction for the L-glutamic acid biosynthesis includes all of CS, PEPC and GDH.

(4) a microorganism of any one of the above (1) to (3) wherein the enzyme catalyzing the reaction branching from the pathway for L-glutamic acid biosynthesis and producing the compound other than L-glutamic acid is  $\alpha$ -ketoglutarate dehydrogenase (abbreviated as "aKGDH" hereinafter).

(5) a microorganism of any one of the above (1) to (4) which is *Enterobacter agglomerans* or *Serratia liquefaciens*; and

(6) a method for producing L-glutamic acid which comprises culturing the microorganism as defined in any one of the above (1) to (5) in a liquid culture medium to produce and accumulate L-glutamic acid in the culture medium, and collecting the L-glutamic acid from the culture medium.

[0007] Because the microorganism of the present invention have a high ability to produce L-glutamic acid, it is considered to be possible to impart a further higher production ability to the microorganism by using the breeding techniques previously known for the coryneform L-glutamic acid-producing bacteria and the like, and it is expected to contribute

to development of a more inexpensive and more efficient method for producing L-glutamic acid by appropriately selecting culture conditions and the like

#### BRIEF EXPLANATION OF THE DRAWINGS

- 5 [0008] Figure 1 shows construction of a plasmid pMWCPG having a *gltA* gene, a *ppc* gene and a *gdhA* gene  
 [0009] Figure 2 shows construction of a plasmid pSTVG having the *gdhA* gene  
 [0010] Figure 3 shows construction of a plasmid RSF-Tet having a replication origin of a wide-host-range plasmid RSF1010 and a tetracycline resistance gene  
 10 [0011] Figure 4 shows construction of a plasmid RSFCPG having the replication origin of the wide-host-range plasmid RSF1010, the tetracycline resistance gene, the *gltA* gene, the *ppc* gene and the *gdhA* gene.  
 [0012] Figure 5 shows construction of a plasmid pMWCB having the *gltA* gene.  
 [0013] Figure 6 shows a restriction map of a DNA fragment of pTWVEK101 derived from *Enterobacter agglomerans*  
 [0014] Figure 7 shows comparison of an amino acid sequence deduced from a nucleotide sequence of a *sucA* gene  
 15 derived from *Enterobacter agglomerans* with one derived from *Escherichia coli*. The upper sections: *Enterobacter agglomerans*, the lower sections: *Escherichia coli* (the same shall apply hereinafter).  
 [0015] Figure 8 shows comparison of an amino acid sequence deduced from a nucleotide sequence of a *sucB* gene derived from *Enterobacter agglomerans* with one derived from *Escherichia coli*.  
 [0016] Figure 9 shows comparison of an amino acid sequence deduced from a nucleotide sequence of a *sdhB* gene  
 20 derived from *Enterobacter agglomerans* with one derived from *Escherichia coli*  
 [0017] Figure 10 shows comparison of an amino acid sequence deduced from a nucleotide sequence of a *sucC* gene derived from *Enterobacter agglomerans* with one derived from *Escherichia coli*

#### DETAILED DESCRIPTION OF THE INVENTION

- 25 [0018] The present invention will be explained in detail hereinafter  
 [0019] The microorganism of the present invention is a microorganism belonging to the genus *Enterobacter* or *Serratia*, and having at least one of the following properties:

- 30 (a) the microorganism increases in an activity of an enzyme catalyzing a reaction for L-glutamic acid biosynthesis and  
 (b) the microorganism decreases in or is deficient in an activity of an enzyme catalyzing a reaction branching from a pathway for L-glutamic acid biosynthesis and producing a compound other than L-glutamic acid

- 35 [0020] Such a microorganism can be obtained by using a microorganism belonging to the genus *Enterobacter* or the genus *Serratia* as a parent strain, and imparting the properties of the above (a) and/or (b) to the microorganism. Examples of the microorganism belonging to the genus *Enterobacter* or the genus *Serratia* that can be used as the parent strain are listed below:

40 *Enterobacter agglomerans*  
*Enterobacter aerogenes*  
*Enterobacter amnigenus*  
*Enterobacter asburiae*  
*Enterobacter cloacae*  
 45 *Enterobacter dissolvens*  
*Enterobacter gergoviae*  
*Enterobacter hormaechei*  
*Enterobacter intermedius*  
*Enterobacter nimipressuralis*  
 50 *Enterobacter sakazakii*  
*Enterobacter taylorae*  
*Serratia liquefacience*  
*Serratia ontomophila*  
*Serratia ficaria*  
 55 *Serratia fonticola*  
*Serratia grimesii*  
*Serratia proteamaculans*  
*Serratia odorifera*

*Serratia plymuthica**Serratia rubidaea*

[0021] More preferably, those bacterial strains listed below can be mentioned

*Enterobacter agglomerans* ATCC 12287

*Enterobacter agglomerans* AJ13355

*Serratia liquefacience* ATCC 14460

[0022] The *Enterobacter agglomerans* AJ13355 was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry on February 19, 1998, and received an accession number of FERM P-16644, and then transferred to an international deposition under the Budapest Treaty on January 11, 1999, and received an accession number of FERM BP-6614. The *Enterobacter agglomerans* ATCC 12287, and the *Serratia liquefacience* ATCC 14460 are available from ATCC.

[0023] The *Enterobacter agglomerans* AJ13355 is a strain isolated from soil in Iwata-shi, Shizuoka, Japan.

[0024] Physiological properties of AJ13355 are as follows

- (1) Gram stain: Negative
- (2) Behavior for oxygen: Facultative anaerobe
- (3) Catalase: Negative
- (4) Oxidase: Positive
- (5) Nitrate reduction: Negative
- (6) Voges-Proskauer reaction: Positive
- (7) Methyl Red test: Negative
- (8) Urease: Negative
- (9) Indole production: Positive
- (10) Motility: Present
- (11) Hydrogen sulfide production in TSI culture medium: Slightly active
- (12)  $\beta$ -Galactosidase: Positive
- (13) Sugar assimilability:
  - Arabinose: Positive
  - Sucrose: Positive
  - Lactose: Positive
  - Xylose: Positive
  - Sorbitol: Positive
  - Inositol: Positive
  - Trehalose: Positive
  - Maltose: Positive
  - Melibiose: Positive
  - Adonitol: Negative
  - Raffinose: Positive
  - Salicin: Negative
  - Melibiose: Positive
- (14) Glycerose assimilability: Positive
- (15) Organic acid assimilability:
  - Citric acid: Positive
  - Tartaric acid: Negative
  - Gluconic acid: Positive
  - Acetic acid: Positive
  - Malonic acid: Negative
- (16) Arginine dehydratase: Negative
- (17) Ornithine decarboxylase: Negative
- (18) Lysine decarboxylase: Negative
- (19) Phenylalanine deaminase: Negative

(20) Pigment formation Yellow

(21) Gelatin liquefaction Positive

(22) Growth pH Not good growth at pH 4 good growth at pH 4.5 to 7

(23) Growth temperature Good growth at 25°C good growth at 30°C good growth at 37°C growth possible at 42°C, no growth at 45°C

[0025] From these bacteriological properties, AJ13355 is determined to be *Enterobacter agglomerans*

[0026] In the working examples described hereinafter *Enterobacter agglomerans* ATCC12287, *Enterobacter agglomerans* AJ13355, and *Serratia liquefacience* ATCC14460 were used as starting parent strains for obtaining strains which increase in the activity of the enzyme catalyzing the reactions for the L-glutamic acid biosynthesis, or strains which decrease in or are deficient in the activity of the enzyme catalyzing the reaction branching from the pathway for L-glutamic acid biosynthesis and producing the compound other than L-glutamic acid. However, the sugar metabolism by any of bacteria belonging to the genera *Enterobacter* and *Serratia* is achieved via the Embden-Meyerhof pathway, and pyruvate produced in the pathway is oxidized in the tricarboxylic acid cycle under aerobic conditions. L-Glutamic acid is biosynthesized from  $\alpha$ -ketoglutaric acid which is an intermediate of the tricarboxylic acid cycle by GDH or glutamine synthetase/glutamate synthase. Thus, these microorganisms share the same biosynthetic pathway for L-glutamic acid, and microorganism belonging to the genera *Enterobacter* and *Serratia* are encompassed within a single concept according to the present invention. Therefore, microorganisms belonging to the genera *Enterobacter* and *Serratia* other than species and strains specifically mentioned above also fall within the scope of the present invention.

[0027] The microorganism of the present invention is a microorganism belonging to the genus *Enterobacter* or the genus *Serratia* and having an ability to produce L-glutamic acid. The expression "having an ability to produce L-glutamic acid" as herein used means to have an ability to accumulate L-glutamic acid in a culture medium during cultivation. According to the present invention, the ability to produce L-glutamic acid is imparted by giving either one or both of the following characteristics:

(a) the microorganism increases in the activity of the enzyme catalyzing the reaction for the L-glutamic acid biosynthesis; and

(b) the microorganism decreases in or is deficient in the activity of the enzyme catalyzing the reaction branching from the pathway for L-glutamic acid biosynthesis and producing the compound other than L-glutamic acid

[0028] As examples of the enzyme catalyzing the reaction for L-glutamic acid biosynthesis of microorganisms of the genus *Enterobacter* or *Serratia*, there can be mentioned GDH, glutamine synthetase, glutamate synthase, isocitrate dehydrogenase, aconitate hydratase, CS, PEPC, pyruvate dehydrogenase, pyruvate kinase, enolase, phosphoglyceromutase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase, fructose biphosphate aldolase, phosphofructokinase, glucose phosphate isomerase and the like. Among these enzymes, one or two or three kinds of CS, PEPC and GDH are preferred. As for the microorganism of the present invention, it is further preferred that activities of all of the three kinds of enzymes, CS, PEPC and GDH, are increased. Whether a microorganism increases in an activity of a target enzyme, and degree of the increase of the activity can be determined by measuring the enzyme activity of a bacterial cell extract or a purified fraction, and comparing it with that of a wild type strain or a parent strain.

[0029] The microorganism of the present invention, which belongs to the genus *Enterobacter* or *Serratia*, and increases in the activity of the enzyme catalyzing the reaction for L-glutamic acid biosynthesis, can be obtained as, for example, a variant where mutation has been made in a gene encoding the enzyme or a genetic recombinant strain by using any of the microorganisms mentioned above as a starting parent strain.

[0030] To enhance the activity of CS, PEPC or GDH, for example, a gene encoding CS, PEPC or GDH can be cloned in a suitable plasmid, and the aforementioned starting parent strain as a host can be transformed with the resulting plasmid. This can increase the copy number of each of the genes encoding CS, PEPC and GDH (hereinafter abbreviated as "gltA gene", "ppc gene", and "gdhA gene" respectively), and as a result the activities of CS, PEPC and GDH can be increased.

[0031] One or two or three kinds selected from the cloned gltA gene, ppc gene and gdhA gene in any combination are introduced into the starting parent strain mentioned above. When two or three kinds of the genes are introduced, either the two or three kinds of the genes are cloned in one kind of plasmid, and introduced into the host, or they are separately cloned in two or three kinds of plasmids that can exist in the same host, and introduced into the host.

[0032] The plasmid is not particularly limited so long as it can autonomously replicate in a microorganism belonging to the genus *Enterobacter* or *Serratia*. Examples of the plasmid include, for example, pUC19, pUC18, pBR322, pHSG299, pHSG298, pHSG399, pHSG398, RSF1010, pMW119, pMW118, pMW219, pMW218 and the like. Other than these plasmids, phage DNA vectors can also be utilized.

[0033] Transformation can be achieved by, for example, the method of D. M. Morrison (Methods in Enzymology 68,

326 (1979)) the method by increasing permeability of recipient cells for DNA with calcium chloride (Mandel M. and Higa A. J. Mol. Biol. 53:159 (1970)) or the like

[0034] The activities of CS, PEPC and GDH can also be increased by using multiple copies of the *gltA* gene, the *ppc* gene and/or the *gdhA* gene present on the chromosome DNA of the starting parent strain as a host. In order to introduce multiple copies of the *gltA* gene, the *ppc* gene and/or the *gdhA* gene into a chromosome DNA of a microorganism belonging to the genus *Enterobacter* or *Serratia*, sequences present on chromosome DNA in a multiple copy number such as repetitive DNA, and inverted repeats present at an end of transposition factors can be utilized. Alternatively, multiple copies of the genes can also be introduced into a chromosome DNA by utilizing transposition of transposons carrying the *gltA* gene, the *ppc* gene, or the *gdhA* gene. These techniques can increase the copy number of the *gltA* gene, the *ppc* gene, and the *gdhA* gene in transformant cells, and as a result increase the activities of CS, PEPC and GDH.

[0035] Any organisms can be used as a source of the *gltA* gene, the *ppc* gene and the *gdhA* gene used for increasing copy numbers, so long as the organisms have the CS, PEPC and GDH activities. Among such organisms, bacteria, i.e., prokaryotes, such as those bacteria belonging to the genera *Enterobacter*, *Klebsiella*, *Erwinia*, *Pantoea*, *Serratia*, *Escherichia*, *Corynebacterium*, *Brevibacterium*, and *Bacillus* are preferred. As a specific example, *Escherichia coli* can be mentioned. The *gltA* gene, the *ppc* gene and the *gdhA* gene can be obtained from a chromosome DNA of such microorganisms as mentioned above.

[0036] The *gltA* gene, the *ppc* gene and the *gdhA* gene can each be obtained from a chromosome DNA of any of the aforementioned microorganisms by isolating a DNA fragment complementing auxotrophy of a variant strain lacking the CS, PEPC or GDH activity. Alternatively, because the nucleotide sequences of these genes of bacteria of the genus *Escherichia* or *Corynebacterium* have already been elucidated (Biochemistry Vol. 22, pp 5243-5249, 1983; J. Biochem. Vol. 95, pp 909-916, 1984; Gene Vol. 27, pp 193-199, 1984; Microbiology Vol. 140, pp 1617-1828, 1994; Mol. Gen. Genet. Vol. 218, pp 330-339, 1989; and Molecular Microbiology Vol. 6, pp 317-326, 1992), the genes can be obtained by PCR using primers synthesized based on each of the elucidated nucleotide sequences, and the chromosome DNA as a template.

[0037] The activity of CS, PEPC or GDH can also be increased by, other than by the gene amplification mentioned above, enhancing expression of the *gltA* gene, the *ppc* gene or the *gdhA* gene. For example, the expression is enhanced by replacing the promoter of the *gltA* gene, the *ppc* gene, or the *gdhA* gene with another stronger promoter. Examples of such a strong promoter include, for example, a *lac* promoter, a *trp* promoter, a *trc* promoter, a *tac* promoter, a  $P_R$  promoter and a  $P_L$  promoter of lambda phage and the like. The *gltA* gene, the *ppc* gene, or the *gdhA* gene of which promoter has been substituted is cloned into a plasmid and introduced into a host microorganism, or introduced into a chromosome DNA of host microorganism using a repetitive DNA, inverted repeat, transposon or the like.

[0038] The activities of CS, PEPC or GDH can also be increased by replacing the promoter of the *gltA* gene, the *ppc* gene, or the *gdhA* gene on a chromosome with another stronger promoter (see WO87/03006, and Japanese Patent Application Laid-Open (KOKAI) No. 61-268183(1986)), or inserting a strong promoter at the upstream of each coding sequence of the genes (see Gene, 29, pp 231-241, 1984). Specifically, these are achieved by homologous recombination between the *gltA* gene, the *ppc* gene, or the *gdhA* gene of which promoter is replaced with a stronger promoter or DNA containing a part of them, and a corresponding gene on the chromosome.

[0039] Specific examples of the microorganism belonging to the genus *Enterobacter* or *Serratia* of which CS, PEPC or GDH activity is increased include, for example, *Enterobacter agglomerans* ATCC12287/RSFCPG, *Enterobacter agglomerans* AJ13355/RSFCPG, and *Serratia liquefacience* ATCC14460/RSFCPG.

[0040] Examples of the enzyme catalyzing the reaction branching from the pathway of L-glutamic acid biosynthesis and producing the compound other than L-glutamic acid include, for example,  $\alpha$ KGDH, isocitrate lyase, phosphate acetyltransferase, acetate kinase, acetohydroxy acid synthase, acetolactate synthase, formate acetyltransferase, lactate dehydrogenase, glutamate decarboxylase, 1-pyrroline dehydrogenase and the like. Among these enzymes,  $\alpha$ KGDH is preferred.

[0041] In order to obtain such decrease or deficiency of enzyme activity as mentioned above in a microorganism belonging to the genus *Enterobacter* or *Serratia*, a mutation causing the decrease or deficiency of the enzyme activity can be introduced into a gene encoding the enzyme by a conventional mutagenesis technique or genetic engineering technique.

[0042] Examples of the mutagenesis technique include, for example, the method utilizing irradiation of X-ray or ultraviolet light, the method utilizing treatment with a mutagenic agent such as N-methyl-N'-nitro-N-nitrosoguanidine and the like. The site of gene to which a mutation is introduced may be a coding region, encoding an enzymic protein, or an expression regulatory region such as a promoter.

[0043] Examples of the genetic engineering technique include, for example, genetic recombination, genetic transduction, cell fusion and the like. For example, a drug resistance gene is inserted into a target gene to produce a functionally inactivated gene (defective gene). Then, this defective gene is introduced into a cell of a microorganism belonging to the genus *Enterobacter* or *Serratia*, and the target gene on a chromosome is replaced with the defective

gene by homologous recombination (gene disruption)

**[0044]** Whether a microorganism decreases in an activity of a target enzyme or is deficient in the activity and degree of the decrease of the activity can be determined by measuring the enzyme activity of a bacteria cell extract or a purified fraction of a candidate strain, and comparing it with that of a wild type strain or a parent strain. The  $\alpha$ KGDH enzymatic activity can be measured by, for example, the method of Reed et al. (L.J. Reed and B.B. Mukherjee, Methods in Enzymology 1969, 13, p. 55-61).

**[0045]** Depending on the target enzyme, a target variant can be selected based on a phenotype of the variant. For example, a variant which is deficient in the  $\alpha$ KGDH activity or decreases in the activity cannot grow on a minimal medium containing glucose, or a minimal medium containing acetic acid or L-glutamic acid as an exclusive carbon source, or shows markedly reduced growth rate therein under aerobic conditions. However, even under the same condition, it can exhibit normal growth by addition of succinic acid or lysine, methionine and diaminopimelate to the minimal medium containing glucose. Based on these phenomena, a variant that is deficient in the  $\alpha$ KGDH activity or decreases in the activity can be selected.

**[0046]** A method for producing a *Brevibacterium lactofermentum* strain lacking the  $\alpha$ KGDH gene based on homologous recombination is detailed in WO95/34672, and a similar method can be used for microorganisms belonging to the genera *Enterobacter* and *Serratia*.

**[0047]** In addition, procedures of genetic cloning, cleavage and ligation of DNA, transformation and the like are detailed in Molecular Cloning, 2nd edition, Cold Spring Harbor Press (1989) and the like.

**[0048]** An example of the variant strain that is deficient in the  $\alpha$ KGDH activity or decreases in the activity obtained as described above is *Enterobacter agglomerans* AJ13356. The *Enterobacter agglomerans* AJ13356 was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry on February 19, 1998, received an accession number of FERM P-16645, and then transferred to an international deposition under the Budapest Treaty on January 11, 1999, and received an accession number of FERM BP-6615.

**[0049]** The microorganism belonging to the genus *Enterobacter* or *Serratia*, and having at least one of the properties (a) and (b) and an ability to produce L-glutamic acid can be cultured in a liquid medium to produce and accumulate L-glutamic acid in the medium.

**[0050]** The culture medium may be an ordinary nutrient medium containing a carbon source, a nitrogen source, and inorganic salts, as well as organic trace nutrients such as amino acids, vitamins and the like, as required. It can be a synthetic medium or a natural medium. Any carbon sources and nitrogen sources can be used for the culture medium so long as they can be utilized by the microorganism to be cultured.

**[0051]** The carbon source may be a saccharide such as glucose, glycerol, fructose, sucrose, maltose, mannose, galactose, starch hydrolysates, molasses and the like. Further, an organic acid such as acetic acid and citric acid may also be used alone or in combination with other carbon sources.

**[0052]** The nitrogen source may be ammonia, ammonium salts such as ammonium sulfate, ammonium carbonate, ammonium chloride, ammonium phosphate, and ammonium acetate, nitrates and the like.

**[0053]** As organic trace nutrients, amino acids, vitamins, fatty acids, nucleic acids, materials containing them such as peptone, casamino acid, yeast extract, and soybean protein decomposition products and the like are used, and when an auxotrophic variant which requires an amino acid or the like for its growth is used, it is necessary to complement the nutrient required.

**[0054]** As the inorganic salt, phosphates, magnesium salts, calcium salts, iron salts, manganese salts and the like are used.

**[0055]** As for the culture conditions, cultivation may be performed under aerobic conditions at a temperature of 20 to 42°C and a pH of 4 to 8. The cultivation can be continued for 10 hours to 4 days to accumulate a considerable amount of L-glutamic acid in the liquid culture medium.

**[0056]** After the completion of the cultivation, L-glutamic acid accumulated in the culture medium may be collected by a known method. For example, it can be isolated by a method comprising concentrating the medium after removing the cells to crystallize the product, ion exchange chromatography or the like.

## Examples

**[0057]** The present invention will be explained more specifically with reference to the following examples.

### (1) Construction of plasmid having *gltA* gene, *ppc* gene and *gdhA* gene

**[0058]** Procedure for construction of a plasmid having a *gltA* gene, a *ppc* gene and a *gdhA* gene will be explained by referring to Figure 1 to Figure 5.

**[0059]** A plasmid pBRGDH having a *gdhA* gene derived from *Escherichia coli* (Japanese Patent Application Laid-

Open (KOKAI) No. 7-203920(1995)) was digested with *HindIII* and *SphI* and the both ends were blunt-ended by a treatment with T4 DNA polymerase. Then, a DNA fragment containing the *gdhA* gene was purified and collected. On the other hand, a plasmid pMWCP having a *gltA* gene and a *ppc* gene derived from *Escherichia coli* (WO97/02294) was digested with *XbaI*, and the both ends were blunt-ended by a treatment with T4 DNA polymerase. This was mixed with the DNA fragment having the *gdhA* gene purified above, and ligated with T4 ligase, giving a plasmid pMWCPG which corresponds to the pMWCP further carrying the *gdhA* gene (Figure 1).

[0060] A DNA fragment having the *gdhA* gene obtained by digesting the pBRGDH with *HindIII* and *Sall* was purified and collected, and introduced into the *HindIII*-*Sall* site of a plasmid pSTV29 (purchased from Takara Shuzo) to obtain a plasmid pSTVG (Figure 2).

[0061] At the same time, a product obtained by digesting a plasmid pVIC40 having a replication origin of a wide-host-range plasmid RSF1010 (Japanese Patent Application Laid-Open (KOKAI) No. 8-047397(1996)) with *NotI*, followed by T4 DNA polymerase treatment and *PstI* digestion, and a product obtained by digesting pBR322 with *EcoT141*, followed by T4 DNA polymerase treatment and *PstI* digestion, were mixed and ligated with T4 ligase to obtain a plasmid RSF-Tet having the replication origin of RSF1010 and a tetracycline resistance gene (Figure 3).

[0062] Then, the pMWCPG was digested with *EcoRI* and *PstI*, and a DNA fragment having the *gltA* gene, the *ppc* gene and the *gdhA* gene was purified and collected. Similarly, the RSF-Tet was digested with *EcoRI* and *PstI*, and a DNA fragment having the replication origin of RSF1010 was purified and collected. Those DNA fragments were mixed and ligated with T4 ligase to obtain a plasmid RSFCPG composed of RSF-Tet carrying the *gltA* gene, the *ppc* gene and the *gdhA* gene (Figure 4). Expression of the *gltA* gene, the *ppc* gene and the *gdhA* gene by the resulting plasmid RSFCPG, and expression of the *gdhA* gene by the pSTVG were confirmed based on complementation of auxotrophy of *Escherichia coli* strains lacking the *gltA* gene, the *ppc* gene or the *gdhA* gene, and measurement of each enzyme activity.

[0063] A plasmid having a *gltA* gene derived from *Brevibacterium lactofermentum* was constructed as follows. PCR was performed by using primers having the nucleotide sequences represented in SEQ ID NOS. 6 and 7 selected based on the nucleotide sequence of the *gltA* gene of *Corynebacterium glutamicum* (Microbiology, 140, 1817-1828, 1994), and a chromosome DNA of *Brevibacterium lactofermentum* ATCC 13869 as a template to obtain a *gltA* gene fragment of about 3 kb. This fragment was inserted into a plasmid pHSG399 (purchased from Takara Shuzo) digested with *SmaI* to obtain a plasmid pHSGCB (Figure 5). Then, the pHSGCB was digested with *HindIII*, and an excised *gltA* gene fragment of about 3 kb was inserted into a plasmid pMW218 (purchased from Nippon Gene) digested with *HindIII* to obtain a plasmid pMWCB (Figure 5). Expression of the *gltA* gene by the resulting plasmid pMWCB was confirmed by determination of enzyme activity in the *Enterobacter agglomerans* AJ13355.

(2) Introduction of RSFCPG, pMWCB and pSTVG into *Enterobacter agglomerans* or *Serratia liquefacience*, and evaluation of L-glutamic acid productivity

[0064] The *Enterobacter agglomerans* ATCC 12287, the *Enterobacter agglomerans* AJ13355 and the *Serratia liquefacience* ATCC 14460 were transformed with the RSFCPG, pMWCB and pSTVG by electroporation (Miller J.H., "A Short Course in Bacterial Genetics, Handbook" Cold Spring Harbor Laboratory Press, USA, 1992) to obtain transformants exhibiting tetracycline resistance.

[0065] Each of the resulting transformants and the parent strains was inoculated into 50 ml-volume large size test tube containing 5 ml of a culture medium comprising 40 g/L glucose, 20 g/L ammonium sulfate, 0.5 g/L magnesium sulfate heptahydrate, 2 g/L potassium dihydrogenphosphate, 0.5 g/L sodium chloride, 0.25 g/L calcium chloride heptahydrate, 0.02 g/L ferrous sulfate heptahydrate, 0.02 g/L manganese sulfate tetrahydrate, 0.72 mg/L zinc sulfate dihydrate, 0.64 mg/L copper sulfate pentahydrate, 0.72 mg/L cobalt chloride hexahydrate, 0.4 mg/L boric acid, 1.2 mg/L sodium molybdate dihydrate, 2 g/L yeast extract, and 30 g/L calcium carbonate, and cultured at 37°C with shaking until the glucose contained in the culture medium was consumed. However, as for the AJ13355/pMWCB strain and the AJ13355/pSTVG strain, the cultivation was stopped when about 10 g/L of glucose was consumed, i.e., cultivated for 15 hours like the parent strain AJ13355, because their glucose consumption rates were low. To the culture medium of the transformants, 25 mg/L of tetracycline was added. After the cultivation was completed, L-glutamic acid accumulated in the culture medium was measured. The results are shown in Table 1.

Table 1

Accumulated amount of L-glutamic acid	
Bacterial strain	Accumulated amount of L-glutamic acid
ATCC12287	0.0 g/L
ATCC12287/RSFCPG	6.1



Table 1 (continued)

Accumulated amount of L-glutamic acid	
Bacterial strain	Accumulated amount of L-glutamic acid
AJ13355	0.0
AJ13355/RSFCPG	3.3
AJ13355/pMWCB	0.8
AJ13355/pSTVG	0.8
ATCC14460	0.0
ATCC14460/RSFCPG	2.8
Culture medium alone	0.2

[0066] While the *Enterobacter agglomerans* ATCC12287, the *Enterobacter agglomerans* AJ13355 and the *Serratia liquefaciens* ATCC14460 did not accumulate L-glutamic acid, the strains whose CS, PEPC and GDH activities were amplified by introducing RSFCPG accumulated 6.1 g/L, 3.3 g/L, and 2.8 g/L of L-glutamic acid, respectively. The AJ13355 strain of which CS activity alone was amplified accumulated 0.8 g/L of L-glutamic acid, and the strain of which GDH activity alone was amplified also accumulated 0.8 g/L of L-glutamic acid.

(3) Cloning of  $\alpha$ KGDH gene (referred to as "sucAB" hereinafter) of *Enterobacter agglomerans* AJ13355

[0067] The *sucAB* gene of the *Enterobacter agglomerans* AJ13355 was cloned by selecting a DNA fragment complementing acetate non-assimilation of an *Escherichia coli* strain lacking the  $\alpha$ KGDH-E1 subunit gene (referred to as "sucA" hereinafter) from the chromosome DNA of the *Enterobacter agglomerans* AJ13355.

[0068] The chromosome DNA of the *Enterobacter agglomerans* AJ13355 strain was isolated by the same method as conventionally used for extracting chromosome DNA from *Escherichia coli* (Seibutsu Kogaku Jikken-sho (Textbook of Bioengineering Experiments), Ed. by the Society of Fermentation and Bioengineering, Japan, p.97-98, Baifukan, 1992). The pTWV228 used as the vector (ampicillin resistant) was a marketed product from Takara Shuzo.

[0069] Products obtained by digesting the chromosome DNA of the AJ13355 strain with *Eco*T221 and products obtained by digesting the pTWV228 with *Pst*I were ligated by T4 ligase, and the *Escherichia coli* JRG465 lacking *sucA* (Herbert J. et al., Mol. Gen. Genetics, 1969, 105, p.182) was transformed with them. Strains grown on the acetic acid minimal medium were selected from the transformants obtained as described above, and a plasmid extracted from them was designated as pTWVEK101. The *Escherichia coli* JRG465 carrying the pTWVEK101 recovered the characteristics of acetate non-assimilability as well as auxotrophy for succinic acid or L-lysine and L-methionine. This suggests that the pTWVEK101 contains the *sucA* gene of *Enterobacter agglomerans*.

[0070] A restriction map of *Enterobacter agglomerans*-derived DNA fragment of pTWVEK101 is shown in Figure 6. The result of nucleotide sequencing of the hatched portion in Figure 6 is shown in SEQ ID NO. 1. In this sequence, two full length ORFs and two nucleotide sequences considered as partial sequences of ORFs were found. Amino acid sequences that can be encoded by these ORFs and the partial sequences thereof are shown in SEQ ID NOS. 2 to 5 in order from the 5' ends. As a result of homology analysis of these sequences, it was found that the portion of which nucleotide sequence had been determined contained a 3' partial sequence of succinate dehydrogenase iron-sulfur protein gene (*sdhB*), full length *sucA* and  $\alpha$ KGDH-E2 subunit gene (*sucB* gene), and 5' partial sequence of succinyl-CoA synthetase  $\beta$  subunit gene (*sucC* gene). Comparison of the amino acid sequences deduced from these nucleotide sequences with those of *Escherichia coli* (Eur. J. Biochem., 141, 351-359 (1984); Eur. J. Biochem., 141, 361-374 (1984), and Biochemistry, 24, 6245-6252 (1985)) is shown in Figures 7 to 9. As shown by these results, the amino acid sequences exhibited markedly high homology. It was also found that a cluster of *sdhB-sucA-sucB-sucC* is formed on the *Enterobacter agglomerans* chromosome like *Escherichia coli* (Eur. J. Biochem., 141, 351-359 (1984); Eur. J. Biochem., 141, 361-374 (1984) and Biochemistry, 24, 6245-6252 (1985)).

(4) Acquisition of strain deficient in  $\alpha$ KGDH derived from *Enterobacter agglomerans* AJ13355

[0071] Using the *sucAB* gene of *Enterobacter agglomerans* obtained as described above, a strain lacking  $\alpha$ KGDH of *Enterobacter agglomerans* was obtained by homologous recombination.

[0072] First, pTWVEK101 was digested with *Bgl*II to remove the C-terminus region corresponding to about half of the *sucA* gene and the full length of the *sucB* gene. To this site, a chloramphenicol resistance gene fragment cut out from the pHSG399 (Takara Shuzo) with *Acc*I was then inserted. The region of *sdhB- $\Delta$ sucAB::Cm<sup>r</sup>-sucC* obtained above was cut out with *Afl*III and *Sac*I. The resulting DNA fragment was used to transform the *Enterobacter agglomerans*.

AJ13355 strain by electroporation to obtain a chloramphenicol resistant strain and thus a *Enterobacter agglomerans* AJ13356 strain lacking the *sucAB* gene where the *sucAB* gene on the chromosome was replaced by *sucAB*<sup>cmr</sup> was obtained

[0073] To confirm that the AJ13356 strain obtained as described above was deficient in the  $\alpha$ KGDH activity its enzymatic activity was determined by the method of Reed (L. L. Reed and B. B. Mukherjee, Methods in Enzymology 1969, 13, p 55-61). As a result the  $\alpha$ KGDH activity could not be detected in the AJ13356 strain as shown in Table 2 and thus it was confirmed that the strain lacked the *sucAB* as desired

Table 2

$\alpha$ KGDH activity	
Bacterial strain	$\alpha$ KGDH activity ( $\Delta$ ABS/min/mg protein)
AJ13355	0.481
AJ13356	<0.0001

(5) Evaluation of L-glutamic acid productivity of *Enterobacter agglomerans* strain deficient in  $\alpha$ KGDH

[0074] Each of the AJ13355 and AJ13356 strains was inoculated into a 500 ml-volume flask containing 20 ml of a culture medium comprising 40 g/L glucose, 20 g/L ammonium sulfate, 0.5 g/L magnesium sulfate heptahydrate, 2 g/L potassium dihydrogenphosphate, 0.5 g/L sodium chloride, 0.25 g/L calcium chloride heptahydrate, 0.02 g/L ferrous sulfate heptahydrate, 0.02 g/L manganese sulfate tetrahydrate, 0.72 mg/L zinc sulfate dihydrate, 0.64 mg/L copper sulfate pentahydrate, 0.72 mg/L cobalt chloride hexahydrate, 0.4 mg/L boric acid, 1.2 mg/L sodium molybdate dihydrate, 2 g/L yeast extract, 30 g/L calcium carbonate, 200 mg/L L-lysine monohydrochloride, 200 mg/L L-methionine and 200 mg/L DL- $\alpha,\epsilon$ -diaminopimelic acid (DAP), and cultured at 37°C with shaking until the glucose contained in the culture medium was consumed. After the cultivation was completed, L-glutamic acid and  $\alpha$ -ketoglutaric acid (abbreviated as " $\alpha$ KG" hereinafter) accumulated in the culture medium were measured. The results are shown in Table 3.

Table 3

Accumulated amounts of L-glutamic acid and $\alpha$ KG		
Bacterial strain	Accumulated amount of L-glutamic acid	Accumulated amount of $\alpha$ KG
AJ13355	0.0 g/L	0.0 g/L
AJ13356	1.5	3.2

[0075] The AJ13356 strain deficient in the  $\alpha$ KGDH activity accumulated 1.5 g/L of L-glutamic acid, and simultaneously accumulated 3.2 g/L of  $\alpha$ KG.

(6) Introduction of RSFCPG into *Enterobacter agglomerans* strain lacking  $\alpha$ KGDH and evaluation of L-glutamic acid productivity

[0076] The AJ13356 strain was transformed with the RSFCPG, and the resulting strain introduced with the RSFCPG, AJ13356/RSFCPG, was inoculated into a 500 ml-volume flask containing 20 ml of a culture medium comprising 40 g/L glucose, 20 g/L ammonium sulfate, 0.5 g/L magnesium sulfate heptahydrate, 2 g/L potassium dihydrogenphosphate, 0.5 g/L sodium chloride, 0.25 g/L calcium chloride heptahydrate, 0.02 g/L ferrous sulfate heptahydrate, 0.02 g/L manganese sulfate tetrahydrate, 0.72 mg/L zinc sulfate dihydrate, 0.64 mg/L copper sulfate pentahydrate, 0.72 mg/L cobalt chloride hexahydrate, 0.4 mg/L boric acid, 1.2 mg/L sodium molybdate dihydrate, 2 g/L yeast extract, 25 mg/L tetracycline, 30 g/L calcium carbonate, 200 mg/L L-lysine monohydrochloride, 200 mg/L L-methionine and 200 mg/L DL- $\alpha,\epsilon$ -DAP, and cultured at 37°C with shaking until the glucose contained in the culture medium was consumed. After the cultivation was completed, L-glutamic acid and  $\alpha$ KG accumulated in the culture medium were measured. The results are shown in Table 4.

Table 4

Accumulated amounts of L-glutamic acid and $\alpha$ KG		
Bacterial strain	Accumulated amount of L-glutamic acid	Accumulated amount of $\alpha$ KG
AJ13356	1.4 g/L	2.9 g/L

EP 0 952 221 A2

Table 4 (continued)

Accumulated amounts of L-glutamic acid and $\alpha$ KG		
Bacterial strain	Accumulated amount of L-glutamic acid	Accumulated amount of $\alpha$ KG
AJ13356/RSFCPG	5.1	0.0

[0077] In the strain of which CS, PEPC and GDH activities were amplified by the introduction of RSFCPG, the accumulated amount of  $\alpha$ KG was reduced, and the accumulated amount of L-glutamic acid was further improved.

Annex to the description

[0078]

5

10

15

20

25

30

35

40

45

50

55

## SEQUENCE LISTING

5 <110> Ajinomoto Co., Inc.  
 <120> L-Glutamic acid-producing bacterium and method for producing L-glutamic acid  
 <130> EPA-53126  
 <150> JP 10-69068  
 10 <151> 1998-03-18  
 <150> JP 10-297129  
 <151> 1998-10-19  
 <160> 7  
 15 <170> PatentIn Ver. 2.0  
  
 <210> 1  
 <211> 4556  
 20 <212> DNA  
 <213> Enterobacter agglomerans  
 <220>  
 <221> CDS  
 <222> (2)..(121)  
 25 <220>  
 <221> CDS  
 <222> (322)..(3129)  
 <220>  
 30 <221> CDS  
 <222> (3145)..(4368)  
 <220>  
 <221> CDS  
 <222> (4437)..(4556)  
 35 <400> 1  
 t gca ttc agc gtt ttc cgc tgt cac agc atc atg aac tgt gta agt gtt 49  
 Ala Phe Ser Val Phe Arg Cys His Ser Ile Met Asn Cys Val Ser Val  
 1 5 10 15  
 40 tgt cct aaa ggg cta aac ccg acg cgc gct atc gcc cac att aag tgg 97  
 Cys Pro Lys Gly Leu Asn Pro Thr Arg Ala Ile Gly His Ile Lys Ser  
 20 25 30  
 atg ctg ctg caa cgc agc gcg tagttatacc accgggaacc tcaggttccc 148  
 45 Met Leu Leu Gln Arg Ser Ala  
 35  
 ggtatitttac ggaagcctct gtaaacgcgg tcccaaccac gtttacaag gtteccctac 208  
 gggccggggcg cgcgctgcgc acagtgtcgc tatcgctgaa ctcaactacgg caaacgggga 268  
 50 aagcggcaac aaatgaaacc tcaaaaaagc ataacattgc ttaagggatc aca atg 324  
 Met  
 1  
 cag aac agc ggc atg aag ccc tgg ctg gac tcc tcc tgg ctg gcc ggc 372  
 55 Gln Asn Ser Ala Met Lys Pro Trp Leu Asp Ser Ser Trp Leu Ala Gly  
 5 10 15

## EP 0 952 221 A2

	gcg aat cag tct tac ata gag caa ctc tat gag gat ttc ctg acc gat	420
	Ala Asn Gln Ser Tyr Ile Glu Gln Leu Tyr Glu Asp Phe Leu Thr Asp	
	20 25 30	
5	cct gac tct gtg gat gca gtg tgg cgc tgc atg ttc caa cag tta cca	468
	Pro Asp Ser Val Asp Ala Val Trp Arg Ser Met Phe Gln Gln Leu Pro	
	35 40 45	
10	ggc acg gga gtg aaa cct gag cag ttc cac tcc gca act cgc gaa tat	516
	Gly Thr Gly Val Lys Pro Glu Gln Phe His Ser Ala Thr Arg Glu Tyr	
	50 55 60 65	
	ttc cgt cgc ctg gcg aaa gac gca tct cgt tac acc tcc tca gtt acc	564
	Phe Arg Arg Leu Lys Asp Ala Ser Arg Tyr Thr Ser Ser Val Thr	
	70 75 80	
15	gat ccg gca acc aac tcc aaa caa gtg aaa gtg ctg cag ctg att aac	612
	Asp Pro Ala Thr Asn Ser Lys Gln Val Lys Val Leu Gln Leu Ile Asn	
	85 90 95	
20	gcg ttt cgt ttc cgc gga cat cag gaa gca aat ctc gat ccg ctt ggc	660
	Ala Phe Arg Phe Arg Gly His Gln Glu Ala Asn Leu Asp Pro Leu Gly	
	100 105 110	
	ctg tgg aaa cag gac cgc gtt gcc gat ctc gat cct gcc ttt cac gat	708
	Leu Trp Lys Gln Asp Arg Val Ala Asp Leu Asp Pro Ala Phe His Asp	
25	115 120 125	
	ctg acc gac gcc gat ttt cag gaa agc ttt aac gta ggt tct ttt gcc	756
	Leu Thr Asp Ala Asp Phe Gln Glu Ser Phe Asn Val Gly Ser Phe Ala	
	130 135 140 145	
30	att ggc aaa gaa acc atg aag ctg gcc gat ctg ttc gac gcg ctg aag	804
	Ile Gly Lys Glu Thr Met Lys Leu Ala Asp Leu Phe Asp Ala Leu Lys	
	150 155 160	
	cag acc tac tgt ggc tgc att ggt gca gag tat atg cac atc aat aac	852
	Gln Thr Tyr Cys Gly Ser Ile Gly Ala Glu Tyr Met His Ile Asn Asn	
35	165 170 175	
	acc gaa gag aaa cgc tgg atc cag cag cgt atc gaa tcc ggt gcg agc	900
	Thr Glu Glu Lys Arg Trp Ile Gln Gln Arg Ile Glu Ser Gly Ala Ser	
	180 185 190	
40	cag acg tca ttc agt ggc gaa gag aaa aaa ggt ttc ctg aaa gag ctg	948
	Gln Thr Ser Phe Ser Gly Glu Glu Lys Lys Gly Phe Leu Lys Glu Leu	
	195 200 205	
	acc gcg gca gaa ggg ctg gaa aaa tat ctg ggc gcg aaa ttc ccg ggt	996
45	Thr Ala Ala Glu Gly Leu Glu Lys Tyr Leu Gly Ala Lys Phe Pro Gly	
	210 215 220 225	
	gca aaa cgt ttc tgc ctg gaa ggc ggt gat gcg ctg gtg ccg atg ctg	1044
	Ala Lys Arg Phe Ser Leu Glu Gly Gly Asp Ala Leu Val Pro Met Leu	
50	230 235 240	
	cgc gag atg att cgt cat gcg ggc aaa agc ggc aca cgt gaa gtg gla	1092
	Arg Glu Met Ile Arg His Ala Gly Lys Ser Gly Thr Arg Glu Val Val	
	245 250 255	
55	ctg ggg atg gcg cac cgt ggc cgt ctt aac gta ctg att aac gta ctg	1140
	Leu Gly Met Ala His Arg Gly Arg Leu Asn Val Leu Ile Asn Val Leu	

## EP 0 952 221 A2

	260	265	270	
	ggt aaa aag cca cag gat ctg ttc gac gaa ttc tcc ggt aaa cac aaa	1188		
	Gly Lys Lys Pro Gln Asp Leu Phe Asp Glu Phe Ser Gly Lys His Lys			
5	275	280	285	
	gag cat ctg ggc acc ggt gat gtg aag tat cac atg ggc ttc tct tcc	1236		
	Glu His Leu Gly Thr Gly Asp Val Lys Tyr His Met Gly Phe Ser Ser			
	290	295	300	305
10	gat att gaa acc gaa ggt ggt ctg gtg cat ctg gcg ctg gcg ttt aac	1284		
	Asp Ile Glu Thr Glu Gly Gly Leu Val His Leu Ala Leu Ala Phe Asn			
	310	315	320	
	ccg tct cac ctg gaa att gtc agc ccg gtg gtc atg gga tcc gta cgt	1332		
15	Pro Ser His Leu Glu Ile Val Ser Pro Val Val Met Gly Ser Val Arg			
	325	330	335	
	gca cgt ctc gat cgt ctg gcc gaa ccg gtc agc aat aaa gtg ttg cct	1380		
	Ala Arg Leu Asp Arg Leu Ala Glu Pro Val Ser Asn Lys Val Leu Pro			
	340	345	350	
20	atc acc att cac ggt gat gcg gcg gtg att ggt cag ggc gtg gtt cag	1428		
	Ile Thr Ile His Gly Asp Ala Ala Val Ile Gly Gln Gly Val Val Gln			
	355	360	365	
	gaa acc ctg aac atg tct cag gcg cgc ggc tac gaa gtg ggc ggc acg	1476		
25	Glu Thr Leu Asn Met Ser Gln Ala Arg Gly Tyr Glu Val Gly Gly Thr			
	370	375	380	385
	gta cgt atc gtc att aac aac cag gtt ggt ttt acc acc tcc aac ccg	1524		
	Val Arg Ile Val Ile Asn Asn Gln Val Gly Phe Thr Thr Ser Asn Pro			
	390	395	400	
30	aaa gat gcg cgt tca acc ccg tac tgt act gac atc ggc aag atg gtg	1572		
	Lys Asp Ala Arg Ser Thr Pro Tyr Cys Thr Asp Ile Gly Lys Met Val			
	405	410	415	
	ctg gca ccg att ttc cac gtc aat gct gac gat ccg gaa gcg gtg gcc	1620		
35	Leu Ala Pro Ile Phe His Val Asn Ala Asp Asp Pro Glu Ala Val Ala			
	420	425	430	
	ttt gtt acc cgc ctg gcg ctg gac tat cgc aac acc ttc aaa cgc gat	1668		
	Phe Val Thr Arg Leu Ala Leu Asp Tyr Arg Asn Thr Phe Lys Arg Asp			
40	435	440	445	
	gtg ttt atc gat ctg gtg tgc tat cgc cgt cat ggt cac aac gag gcg	1716		
	Val Phe Ile Asp Leu Val Cys Tyr Arg Arg His Gly His Asn Glu Ala			
	450	455	460	465
45	gat gag cca agt gct acc cag ccg ttg atg tac cag aaa atc aaa aag	1764		
	Asp Glu Pro Ser Ala Thr Gln Pro Leu Met Tyr Gln Lys Ile Lys Lys			
	470	475	480	
	cat ccg acg ccg cgt aaa att tac gcc gat cgt ctg gaa ggc gaa ggt	1812		
50	His Pro Thr Pro Arg Lys Ile Tyr Ala Asp Arg Leu Glu Gly Glu Gly			
	485	490	495	
	gtc gcg tcc cag gaa gat gcc acc gag atg gtg aac ctg tac cgc gat	1860		
	Val Ala Ser Gln Glu Asp Ala Thr Glu Met Val Asn Leu Tyr Arg Asp			
	500	505	510	
55	gcg ctc gat gcg ggc gaa tgc gtg gtg ccg gaa tgg cgt ccg atg agc	1908		

EP 0 952 221 A2

	Ala Leu Asp Ala Gly Glu Cys Val Val Pro Glu Trp Arg Pro Met Ser	
	515 520 525	
5	ctg cac tcc ttc acg tgg tgg cct tat ctg aac cac gaa tgg gat gag	1956
	Leu His Ser Phe Thr Trp Ser Pro Tyr Leu Asn His Glu Trp Asp Glu	
	530 535 540 545	
	cct tat ccg gca cag gtt gac atg aaa cgc ctg aag gaa ctg gca ttg	2004
	Pro Tyr Pro Ala Gln Val Asp Met Lys Arg Leu Lys Glu Leu Ala Leu	
10	550 555 560	
	cgt atc agc cag gtc cct gag cag att gaa gtg cag tgg cgc gtg gcc	2052
	Arg Ile Ser Gln Val Pro Glu Gln Ile Glu Val Gln Ser Arg Val Ala	
	565 570 575	
15	aag atc tat aac gat cgc aag ctg atg gcc gaa ggc gag aaa gcg ttc	2100
	Lys Ile Tyr Asn Asp Arg Lys Leu Met Ala Glu Gly Glu Lys Ala Phe	
	580 585 590	
	gac tgg ggc ggt gcc gag aat ctg gcg tac gcc acg ctg gtg gat gaa	2148
20	Asp Trp Gly Gly Ala Glu Asn Leu Ala Tyr Ala Thr Leu Val Asp Glu	
	595 600 605	
	ggt att ccg gtt cgc ctc tgg ggt gaa gac tcc ggt cgt gga acc ttc	2196
	Gly Ile Pro Val Arg Leu Ser Gly Glu Asp Ser Gly Arg Gly Thr Phe	
	610 615 620 625	
25	ttc cat cgc cac gcg gtc gtg cac aac cag gct aac ggt tca acc tat	2244
	Phe His Arg His Ala Val Val His Asn Gln Ala Asn Gly Ser Thr Tyr	
	630 635 640	
	acg ccg ctg cac cat att cat aac agc cag ggc gag ttc aaa gtc tgg	2292
30	Thr Pro Leu His His Ile His Asn Ser Gln Gly Glu Phe Lys Val Trp	
	645 650 655	
	gat tgg gtg ctg tct gaa gaa gcg gtg ctg gcg ttt gaa tac ggt tac	2340
	Asp Ser Val Leu Ser Glu Glu Ala Val Leu Ala Phe Glu Tyr Gly Tyr	
	660 665 670	
35	gcc acg gct gag ccg cgc gtg ctg acc atc tgg gaa gcg cag ttt ggt	2388
	Ala Thr Ala Glu Pro Arg Val Leu Thr Ile Trp Glu Ala Gln Phe Gly	
	675 680 685	
	gac ttt gcc aac ggt gct cag gtg gtg att gac cag ttc atc agc tct	2436
40	Asp Phe Ala Asn Gly Ala Gln Val Val Ile Asp Gln Phe Ile Ser Ser	
	690 695 700 705	
	ggc gaa cag aag tgg ggc cgt atg tgt ggc ctg gtg atg ttg ctg ccg	2484
	Gly Glu Gln Lys Trp Gly Arg Met Cys Gly Leu Val Met Leu Leu Pro	
45	710 715 720	
	cat ggc tac gaa ggt cag gga ccg gaa cac tcc tct gcc cgt ctg gaa	2532
	His Gly Tyr Glu Gly Gln Gly Pro Glu His Ser Ser Ala Arg Leu Glu	
	725 730 735	
50	cgc tat ctg caa ctt tgc gcc gag cag aac atg cag gtt tgc gtc ccg	2580
	Arg Tyr Leu Gln Leu Cys Ala Glu Gln Asn Met Gln Val Cys Val Pro	
	740 745 750	
	tgg acg ccg gct cag gtg tat cac atg ctg cgc cgt cag gcg ctg cgc	2628
55	Ser Thr Pro Ala Gln Val Tyr His Met Leu Arg Arg Gln Ala Leu Arg	
	755 760 765	



## EP 0 952 221 A2

	ggg atg cgc cgt ccg ctg gtg gtg atg tcg ccg aag tcg ctg tta cgc	2676
	Gly Met Arg Arg Pro Leu Val Val Met Ser Pro Lys Ser Leu Leu Arg	
	770 775 780 785	
5	cat cca ctg gcg atc tcg tcg ctg gat gaa ctg gca aac ggc agt ttc	2724
	His Pro Leu Ala Ile Ser Ser Leu Asp Glu Leu Ala Asn Gly Ser Phe	
	790 795 800	
10	cag ccg gcc att ggt gag atc gac gat ctg gat ccg cag ggc gtg aaa	2772
	Gln Pro Ala Ile Gly Glu Ile Asp Asp Leu Asp Pro Gln Gly Val Lys	
	805 810 815	
15	cgc gtc gtg ctg tgc tcc ggt aag gtt tac tac gat ctg ctg gaa cag	2820
	Arg Val Val Leu Cys Ser Gly Lys Val Tyr Tyr Asp Leu Leu Glu Gln	
	820 825 830	
20	cgt cgt aaa gac gag aaa acc gat gtt gcc atc gtg cgc atc gaa cag	2868
	Arg Arg Lys Asp Glu Lys Thr Asp Val Ala Ile Val Arg Ile Glu Gln	
	835 840 845	
25	ctt tac ccg ttc ccg cat cag gcg gta cag gaa gca ttg aaa gcc tat	2916
	Leu Tyr Pro Phe Pro His Gln Ala Val Gln Glu Ala Leu Lys Ala Tyr	
	850 855 860 865	
30	tct cac gta cag gac ttt gtc tgg tgc cag gaa gag cct ctg aac cag	2964
	Ser His Val Gln Asp Phe Val Trp Cys Gln Glu Glu Pro Leu Asn Gln	
	870 875 880	
35	ggc gcc tgg tac tgt agc cag cat cat ttc cgt gat gtc gtg ccg ttt	3012
	Gly Ala Trp Tyr Cys Ser Gln His His Phe Arg Asp Val Val Pro Phe	
	885 890 895	
40	ggg gcc acc ctg cgt tat gca ggt cgc ccg gca tcg gct tct ccg gcc	3060
	Gly Ala Thr Leu Arg Tyr Ala Gly Arg Pro Ala Ser Ala Ser Pro Ala	
	900 905 910	
45	gtg ggt tat atg tcc gta cac caa caa cag cag caa gac ctg gtt aat	3108
	Val Gly Tyr Met Ser Val His Gln Gln Gln Gln Gln Asp Leu Val Asn	
	915 920 925	
50	gac gca ctg aac gtc aat taattaaaag gaaagata atg agt agc gta gat	3156
	Asp Ala Leu Asn Val Asn Met Ser Ser Val Asp	
	930 935 1 5	
55	att ctc gtt ccc gac ctg cct gaa tcg gtt gca gat gcc aca gta gca	3204
	Ile Leu Val Pro Asp Leu Pro Glu Ser Val Ala Asp Ala Thr Val Ala	
	10 15 20	
60	acc tgg cac aag aaa cca gcc gat gca gtc agc cgc gat gaa gtc atc	3252
	Thr Trp His Lys Lys Pro Gly Asp Ala Val Ser Arg Asp Glu Val Ile	
	25 30 35	
65	gtc gaa att gaa act gac aaa gtc gtg ctg gaa gtg ccg gca tct gcc	3300
	Val Glu Ile Glu Thr Asp Lys Val Val Leu Glu Val Pro Ala Ser Ala	
	40 45 50	
70	gat ggc gtg ctg gaa gcc gtg ctg gaa gac gaa ggg gca acc gtt acc	3348
	Asp Gly Val Leu Glu Ala Val Leu Glu Asp Glu Gly Ala Thr Val Thr	
	55 60 65	
75	tcc cgc cag atc ctg ggt cgc ctg aaa gaa gcc aac agt gcg ggt aaa	3396
	Ser Arg Gln Ile Leu Gly Arg Leu Lys Glu Gly Asn Ser Ala Gly Lys	

## EP 0 952 221 A2

	70		75		80		85	
	gaa agc agt gcc aaa gcg gaa agc aat gac acc acg cca gcc cag cgt							3447
	Glu Ser Ser Ala Lys Ala Glu Ser Asn Asp Thr Thr Pro Ala Gln Arg							
5		90		95		100		
	cag aca gcg tcg ctt gaa gaa gag agc agc gat gcg ctc agc ccg gcg							3495
	Gln Thr Ala Ser Leu Glu Glu Glu Ser Ser Asp Ala Leu Ser Pro Ala							
		105		110		115		
10	atc cgt cgc ctg att gcg gag cat aat ctt gac gct gcg cag atc aaa							3543
	Ile Arg Arg Leu Ile Ala Glu His Asn Leu Asp Ala Ala Gln Ile Lys							
		120		125		130		
	ggc acc ggc gta ggc gga cgt tta acg cgt gaa gac gtt gaa aaa cat							3591
	Gly Thr Gly Val Gly Gly Arg Leu Thr Arg Glu Asp Val Glu Lys His							
15		135		140		145		
	ctg gcg aac aaa ccg cag gct gag aaa gcc gcc gcg cca gcg gcg ggt							3639
	Leu Ala Asn Lys Pro Gln Ala Glu Lys Ala Ala Pro Ala Ala Gly							
		150		155		160		165
20	gca gca acg gct cag cag cct gtt gcc aac cgc agc gaa aaa cgt gtt							3687
	Ala Ala Thr Ala Gln Gln Pro Val Ala Asn Arg Ser Glu Lys Arg Val							
		170		175		180		
	ccg atg acg cgt tta cgt aag cgc gtc gcg gag cgt ctg ctg gaa gcc							3735
25	Pro Met Thr Arg Leu Arg Lys Arg Val Ala Glu Arg Leu Leu Glu Ala							
		185		190		195		
	aag aac agc acc gcc atg ttg acg acc ttc aac gaa atc aac atg aag							3783
	Lys Asn Ser Thr Ala Met Leu Thr Thr Phe Asn Glu Ile Asn Met Lys							
30		200		205		210		
	ccg att atg gat ctg cgt aag cag tac gcc gat gcg ttc gag aag cgt							3831
	Pro Ile Met Asp Leu Arg Lys Gln Tyr Gly Asp Ala Phe Glu Lys Arg							
		215		220		225		
35	cac ggt gtg cgt ctg gcc ttt atg tct ttc tac atc aag gcc gtg gtc							3879
	His Gly Val Arg Leu Gly Phe Met Ser Phe Tyr Ile Lys Ala Val Val							
		230		235		240		245
	gaa gcg ctg aag cgt tat cca gaa gtc aac gcc tct atc gat gcc gaa							3927
	Glu Ala Leu Lys Arg Tyr Pro Glu Val Asn Ala Ser Ile Asp Gly Glu							
40		250		255		260		
	gac gtg gtg tac cac aac tat ttc gat gtg agt att gcc gtc tct acg							3975
	Asp Val Val Tyr His Asn Tyr Phe Asp Val Ser Ile Ala Val Ser Thr							
		265		270		275		
45	cca cgc gga ctg gtg acg cct gtc ctg cgt gac gtt gat gcg ctg agc							4023
	Pro Arg Gly Leu Val Thr Pro Val Leu Arg Asp Val Asp Ala Leu Ser							
		280		285		290		
	atg gct gac atc gag aag aaa att aaa gaa ctg gca gtg aaa gcc cgt							4071
50	Met Ala Asp Ile Glu Lys Lys Ile Lys Glu Leu Ala Val Lys Gly Arg							
		295		300		305		
	gac gcc aag ctg acg gtt gac gat ctg acg gcc ggt aac ttt acc atc							4119
	Asp Gly Lys Leu Thr Val Asp Asp Leu Thr Gly Gly Asn Phe Thr Ile							
		310		315		320		325
55	acc aac ggt ggt gtg ttc ggt tcg ctg atg tct acg cca atc atc aac							4167

EP 0 952 221 A2

Thr Asn Gly Gly Val Phe Gly Ser Leu Met Ser Thr Pro Ile Ile Asn  
330 335 340  
ccg cca cag agc gcg att ctg ggc atg cac gcc att aaa gat cgt cct 4215  
5 Pro Pro Gln Ser Ala Ile Leu Gly Met His Ala Ile Lys Asp Arg Pro  
345 350 355  
atg gcg gtc aat ggt cag gtt gtg atc ctg cca atg atg tac ctg gct 4263  
Met Ala Val Asn Gly Gln Val Val Ile Leu Pro Met Met Tyr Leu Ala  
10 360 365 370  
ctc tcc tac gat cac cgt tta atc gat ggt cgt gaa tct gtc ggc tat 4311  
Leu Ser Tyr Asp His Arg Leu Ile Asp Gly Arg Glu Ser Val Gly Tyr  
375 380 385  
ctg gtc gcg gtg aaa gag atg ctg gaa gat ccg gcg cgt ctg ctg ctg 4359  
15 Leu Val Ala Val Lys Glu Met Leu Glu Asp Pro Ala Arg Leu Leu Leu  
390 395 400 405  
gat gtc tgattcatca ctgggcacgc gttgcgtgcc caatctcaat actcttttca 4415  
20 Asp Val  
gatctgaatg gatagaacat c atg aac tta cac gaa tac cag gct aaa cag 4466  
Met Asn Leu His Glu Tyr Gln Ala Lys Gln  
1 5 10  
25 ctg ttt gca cgg tat ggc atg cca gca ccg acc ggc tac gcc tgt act 4514  
Leu Phe Ala Arg Tyr Gly Met Pro Ala Pro Thr Gly Tyr Ala Cys Thr  
15 20 25  
aca cca cgt gaa gca gaa gaa gcg gca tcg aaa atc ggt gca 4556  
30 Thr Pro Arg Glu Ala Glu Glu Ala Ala Ser Lys Ile Gly Ala  
30 35 40  
<210> 2  
<211> 39  
35 <212> PRT  
<213> Enterobacter agglomerans  
<400> 2  
Ala Phe Ser Val Phe Arg Cys His Ser Ile Met Asn Cys Val Ser Val  
40 1 5 10 15  
Cys Pro Lys Gly Leu Asn Pro Thr Arg Ala Ile Gly His Ile Lys Ser  
20 25 30  
Met Leu Leu Gln Arg Ser Ala  
45 35  
<210> 3  
<211> 935  
50 <212> PRT  
<213> Enterobacter agglomerans  
<400> 3  
Met Gln Asn Ser Ala Met Lys Pro Trp Leu Asp Ser Ser Trp Leu Ala  
55 1 5 10 15  
Gly Ala Asn Gln Ser Tyr Ile Glu Gln Leu Tyr Glu Asp Phe Leu Thr

EP 0 952 221 A2

		20		25		30
	Asp	Pro	Asp	Ser	Val	Asp
				Ala	Val	Trp
				Arg	Ser	Met
				Phe	Gln	Gln
				Leu		
		35		40		45
5	Pro	Gly	Thr	Gly	Val	Lys
				Pro	Glu	Gln
				Phe	His	Ser
				Ala	Thr	Arg
				Glu		
		50		55		60
	Tyr	Phe	Arg	Arg	Leu	Ala
				Lys	Asp	Ala
				Ser	Arg	Tyr
				Thr	Ser	Ser
				Val		
		65		70		75
				80		
10	Thr	Asp	Pro	Ala	Thr	Asn
				Ser	Lys	Gln
				Val	Lys	Val
				Leu	Gln	Leu
				Ile		
			85		90	
				95		
	Asn	Ala	Phe	Arg	Phe	Arg
				Gly	His	Gln
				Glu	Ala	Asn
				Leu	Asp	Pro
				Leu		
		100		105		110
15	Gly	Leu	Trp	Lys	Gln	Asp
				Arg	Val	Ala
				Asp	Leu	Asp
				Pro	Ala	Phe
				His		
		115		120		125
	Asp	Leu	Thr	Asp	Ala	Asp
				Phe	Gln	Glu
				Ser	Phe	Asn
				Val	Gly	Ser
				Phe		
		130		135		140
20	Ala	Ile	Gly	Lys	Glu	Thr
				Met	Lys	Leu
				Ala	Asp	Leu
				Phe	Asp	Ala
				Leu		
		145		150		155
				160		
	Lys	Gln	Thr	Tyr	Cys	Gly
				Ser	Ile	Gly
				Ala	Glu	Tyr
				Met	His	Ile
				Asn		
			165		170	
				175		
	Asn	Thr	Glu	Glu	Lys	Arg
				Trp	Ile	Gln
				Gln	Arg	Ile
				Glu	Ser	Gly
				Ala		
		180		185		190
25	Ser	Gln	Thr	Ser	Phe	Ser
				Gly	Glu	Glu
				Lys	Lys	Gly
				Phe	Leu	Lys
				Glu		
		195		200		205
	Leu	Thr	Ala	Ala	Glu	Gly
				Leu	Glu	Lys
				Tyr	Leu	Gly
				Ala	Lys	Phe
				Pro		
		210		215		220
30	Gly	Ala	Lys	Arg	Phe	Ser
				Leu	Glu	Gly
				Gly	Gly	Asp
				Ala	Leu	Val
				Pro	Met	
		225		230		235
				240		
	Leu	Arg	Glu	Met	Ile	Arg
				His	Ala	Gly
				Lys	Ser	Gly
				Thr	Arg	Glu
				Val		
		245		250		255
35	Val	Leu	Gly	Met	Ala	His
				Arg	Gly	Arg
				Leu	Asn	Val
				Leu	Ile	Asn
				Val		
		260		265		270
	Leu	Gly	Lys	Lys	Pro	Gln
				Asp	Leu	Phe
				Asp	Glu	Phe
				Ser	Gly	Lys
				His		
		275		280		285
40	Lys	Glu	His	Leu	Gly	Thr
				Gly	Asp	Val
				Lys	Tyr	His
				Met	Gly	Phe
				Ser		
		290		295		300
	Ser	Asp	Ile	Glu	Thr	Glu
				Gly	Gly	Leu
				Val	His	Leu
				Ala	Leu	Ala
				Phe		
		305		310		315
				320		
45	Asn	Pro	Ser	His	Leu	Glu
				Ile	Val	Ser
				Pro	Val	Val
				Met	Gly	Ser
				Val		
		325		330		335
	Arg	Ala	Arg	Leu	Asp	Arg
				Leu	Ala	Glu
				Pro	Val	Ser
				Asn	Lys	Val
				Leu		
		340		345		350
50	Pro	Ile	Thr	Ile	His	Gly
				Asp	Ala	Ala
				Val	Ile	Gly
				Gln	Gly	Val
				Val		
		355		360		365
	Gln	Glu	Thr	Leu	Asn	Met
				Ser	Gln	Ala
				Arg	Gly	Tyr
				Glu	Val	Gly
				Gly		
		370		375		380
55	Thr	Val	Arg	Ile	Val	Ile
				Asn	Asn	Gln
				Val	Gly	Phe
				Thr	Thr	Ser
				Asn		
		385		390		395
				400		

EP 0 952 221 A2

Pro Lys Asp Ala Arg Ser Thr Pro Tyr Cys Thr Asp Ile Gly Lys Met  
405 410 415  
Val Leu Ala Pro Ile Phe His Val Asn Ala Asp Asp Pro Glu Ala Val  
5 420 425 430  
Ala Phe Val Thr Arg Leu Ala Leu Asp Tyr Arg Asn Thr Phe Lys Arg  
435 440 445  
Asp Val Phe Ile Asp Leu Val Cys Tyr Arg Arg His Gly His Asn Glu  
10 450 455 460  
Ala Asp Glu Pro Ser Ala Thr Gln Pro Leu Met Tyr Gln Lys Ile Lys  
465 470 475 480  
Lys His Pro Thr Pro Arg Lys Ile Tyr Ala Asp Arg Leu Glu Gly Glu  
15 485 490 495  
Gly Val Ala Ser Gln Glu Asp Ala Thr Glu Met Val Asn Leu Tyr Arg  
500 505 510  
Asp Ala Leu Asp Ala Gly Glu Cys Val Val Pro Glu Trp Arg Pro Met  
20 515 520 525  
Ser Leu His Ser Phe Thr Trp Ser Pro Tyr Leu Asn His Glu Trp Asp  
530 535 540  
Glu Pro Tyr Pro Ala Gln Val Asp Met Lys Arg Leu Lys Glu Leu Ala  
545 550 555 560  
25 Leu Arg Ile Ser Gln Val Pro Glu Gln Ile Glu Val Gln Ser Arg Val  
565 570 575  
Ala Lys Ile Tyr Asn Asp Arg Lys Leu Met Ala Glu Gly Glu Lys Ala  
580 585 590  
30 Phe Asp Trp Gly Gly Ala Glu Asn Leu Ala Tyr Ala Thr Leu Val Asp  
595 600 605  
Glu Gly Ile Pro Val Arg Leu Ser Gly Glu Asp Ser Gly Arg Gly Thr  
610 615 620  
35 Phe Phe His Arg His Ala Val Val His Asn Gln Ala Asn Gly Ser Thr  
625 630 635 640  
Tyr Thr Pro Leu His His Ile His Asn Ser Gln Gly Glu Phe Lys Val  
645 650 655  
Trp Asp Ser Val Leu Ser Glu Glu Ala Val Leu Ala Phe Glu Tyr Gly  
40 660 665 670  
Tyr Ala Thr Ala Glu Pro Arg Val Leu Thr Ile Trp Glu Ala Gln Phe  
675 680 685  
Gly Asp Phe Ala Asn Gly Ala Gln Val Val Ile Asp Gln Phe Ile Ser  
45 690 695 700  
Ser Gly Glu Gln Lys Trp Gly Arg Met Cys Gly Leu Val Met Leu Leu  
705 710 715 720  
Pro His Gly Tyr Glu Gly Gln Gly Pro Glu His Ser Ser Ala Arg Leu  
50 725 730 735  
Glu Arg Tyr Leu Gln Leu Cys Ala Glu Gln Asn Met Gln Val Cys Val  
740 745 750  
Pro Ser Thr Pro Ala Gln Val Tyr His Met Leu Arg Arg Gln Ala Leu  
755 760 765  
55 Arg Gly Met Arg Arg Pro Leu Val Val Met Ser Pro Lys Ser Leu Leu

EP 0 952 221 A2

770 775 780  
 Arg His Pro Leu Ala Ile Ser Ser Leu Asp Glu Leu Ala Asn Gly Ser  
 785 790 795 800  
 5 Phe Gln Pro Ala Ile Gly Glu Ile Asp Asp Leu Asp Pro Gln Gly Val  
 805 810 815  
 Lys Arg Val Val Leu Cys Ser Gly Lys Val Tyr Tyr Asp Leu Leu Glu  
 820 825 830  
 10 Gln Arg Arg Lys Asp Glu Lys Thr Asp Val Ala Ile Val Arg Ile Glu  
 835 840 845  
 Gln Leu Tyr Pro Phe Pro His Gln Ala Val Gln Glu Ala Leu Lys Ala  
 850 855 860  
 15 Tyr Ser His Val Gln Asp Phe Val Trp Cys Gln Glu Glu Pro Leu Asn  
 865 870 875 880  
 Gln Gly Ala Trp Tyr Cys Ser Gln His His Phe Arg Asp Val Val Pro  
 885 890 895  
 20 Phe Gly Ala Thr Leu Arg Tyr Ala Gly Arg Pro Ala Ser Ala Ser Pro  
 900 905 910  
 Ala Val Gly Tyr Met Ser Val His Gln Gln Gln Gln Gln Asp Leu Val  
 915 920 925  
 25 Asn Asp Ala Leu Asn Val Asn  
 930 935  
 <210> 4  
 <211> 407  
 30 <212> PRT  
 <213> Enterobacter agglomerans  
 <400> 4  
 Met Ser Ser Val Asp Ile Leu Val Pro Asp Leu Pro Glu Ser Val Ala  
 1 5 10 15  
 35 Asp Ala Thr Val Ala Thr Trp His Lys Lys Pro Gly Asp Ala Val Ser  
 20 25 30  
 Arg Asp Glu Val Ile Val Glu Ile Glu Thr Asp Lys Val Val Leu Glu  
 35 40 45  
 40 Val Pro Ala Ser Ala Asp Gly Val Leu Glu Ala Val Leu Glu Asp Glu  
 50 55 60  
 Gly Ala Thr Val Thr Ser Arg Gln Ile Leu Gly Arg Leu Lys Glu Gly  
 65 70 75 80  
 45 Asn Ser Ala Gly Lys Glu Ser Ser Ala Lys Ala Glu Ser Asn Asp Thr  
 85 90 95  
 Thr Pro Ala Gln Arg Gln Thr Ala Ser Leu Glu Glu Glu Ser Ser Asp  
 100 105 110  
 50 Ala Leu Ser Pro Ala Ile Arg Arg Leu Ile Ala Glu His Asn Leu Asp  
 115 120 125  
 Ala Ala Gln Ile Lys Gly Thr Gly Val Gly Gly Arg Leu Thr Arg Glu  
 130 135 140  
 55 Asp Val Glu Lys His Leu Ala Asn Lys Pro Gln Ala Glu Lys Ala Ala  
 145 150 155 160

EP 0 952 221 A2

Ala Pro Ala Ala Gly Ala Ala Thr Ala Gln Gln Pro Val Ala Asn Arg  
155 170 175  
5 Ser Glu Lys Arg Val Pro Met Thr Arg Leu Arg Lys Arg Val Ala Glu  
180 185 190  
Arg Leu Leu Glu Ala Lys Asn Ser Thr Ala Met Leu Thr Thr Phe Asn  
195 200 205  
10 Glu Ile Asn Met Lys Pro Ile Met Asp Leu Arg Lys Gln Tyr Gly Asp  
210 215 220  
Ala Phe Glu Lys Arg His Gly Val Arg Leu Gly Phe Met Ser Phe Tyr  
225 230 235 240  
Ile Lys Ala Val Val Glu Ala Leu Lys Arg Tyr Pro Glu Val Asn Ala  
15 245 250 255  
Ser Ile Asp Gly Glu Asp Val Val Tyr His Asn Tyr Phe Asp Val Ser  
260 265 270  
Ile Ala Val Ser Thr Pro Arg Gly Leu Val Thr Pro Val Leu Arg Asp  
275 280 285  
20 Val Asp Ala Leu Ser Met Ala Asp Ile Glu Lys Lys Ile Lys Glu Leu  
290 295 300  
Ala Val Lys Gly Arg Asp Gly Lys Leu Thr Val Asp Asp Leu Thr Gly  
305 310 315 320  
25 Gly Asn Phe Thr Ile Thr Asn Gly Gly Val Phe Gly Ser Leu Met Ser  
325 330 335  
Thr Pro Ile Ile Asn Pro Pro Gln Ser Ala Ile Leu Gly Met His Ala  
340 345 350  
30 Ile Lys Asp Arg Pro Met Ala Val Asn Gly Gln Val Val Ile Leu Pro  
355 360 365  
Met Met Tyr Leu Ala Leu Ser Tyr Asp His Arg Leu Ile Asp Gly Arg  
370 375 380  
35 Glu Ser Val Gly Tyr Leu Val Ala Val Lys Glu Met Leu Glu Asp Pro  
385 390 395 400  
Ala Arg Leu Leu Leu Asp Val  
405  
40 <210> 5  
<211> 40  
<212> PRT  
<213> Enterobacter agglomerans  
45 <400> 5  
Met Asn Leu His Glu Tyr Gln Ala Lys Gln Leu Phe Ala Arg Tyr Gly  
1 5 10 15  
Met Pro Ala Pro Thr Gly Tyr Ala Cys Thr Thr Pro Arg Glu Ala Glu  
20 25 30  
50 Glu Ala Ala Ser Lys Ile Gly Ala  
35 40  
55 <210> 6  
<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 6

gtcgacaata gccygaatct gttctggtcg

30

<210> 7

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 7

aagcttatcg acgctccccc cccacccgtt

30

#### Claims

1. A microorganism belonging to the genus *Enterobacter* or *Serratia* having the ability to produce L-glutamic acid and having at least one of the following properties:

(a) the microorganism has increased activity of an enzyme catalyzing a reaction in the L-glutamic acid biosynthesis, and

(b) the microorganism has decreased activity or is deficient in an activity of an enzyme catalyzing a reaction branching from the pathway of the L-glutamic acid biosynthesis and producing a compound other than L-glutamic acid

2. A microorganism according to claim 1 wherein the enzyme catalyzing the reaction in the L-glutamic acid biosynthesis is at least one selected from the group consisting of citrate synthase, phosphoenolpyruvate carboxylase, and glutamate dehydrogenase.

3. A microorganism according to claim 2 wherein the enzyme catalyzing the reaction in the L-glutamic acid biosynthesis includes all of citrate synthase, phosphoenolpyruvate carboxylase, and glutamate dehydrogenase

4. A microorganism according to any one of claims 1 to 3 wherein the enzyme catalyzing the reaction branching from the pathway of the L-glutamic acid biosynthesis and producing a compound other than L-glutamic acid is  $\alpha$ -ketoglutarate dehydrogenase

5. A microorganism according to any one of claims 1 to 4 which is *Enterobacter agglomerans* or *Serratia liquefaciens*

6. A method for producing L-glutamic acid which comprises culturing the microorganism as defined in any one of claims 1 to 5 in a liquid culture medium to produce and accumulate L-glutamic acid in the culture medium, and collecting the L-glutamic acid from the culture medium



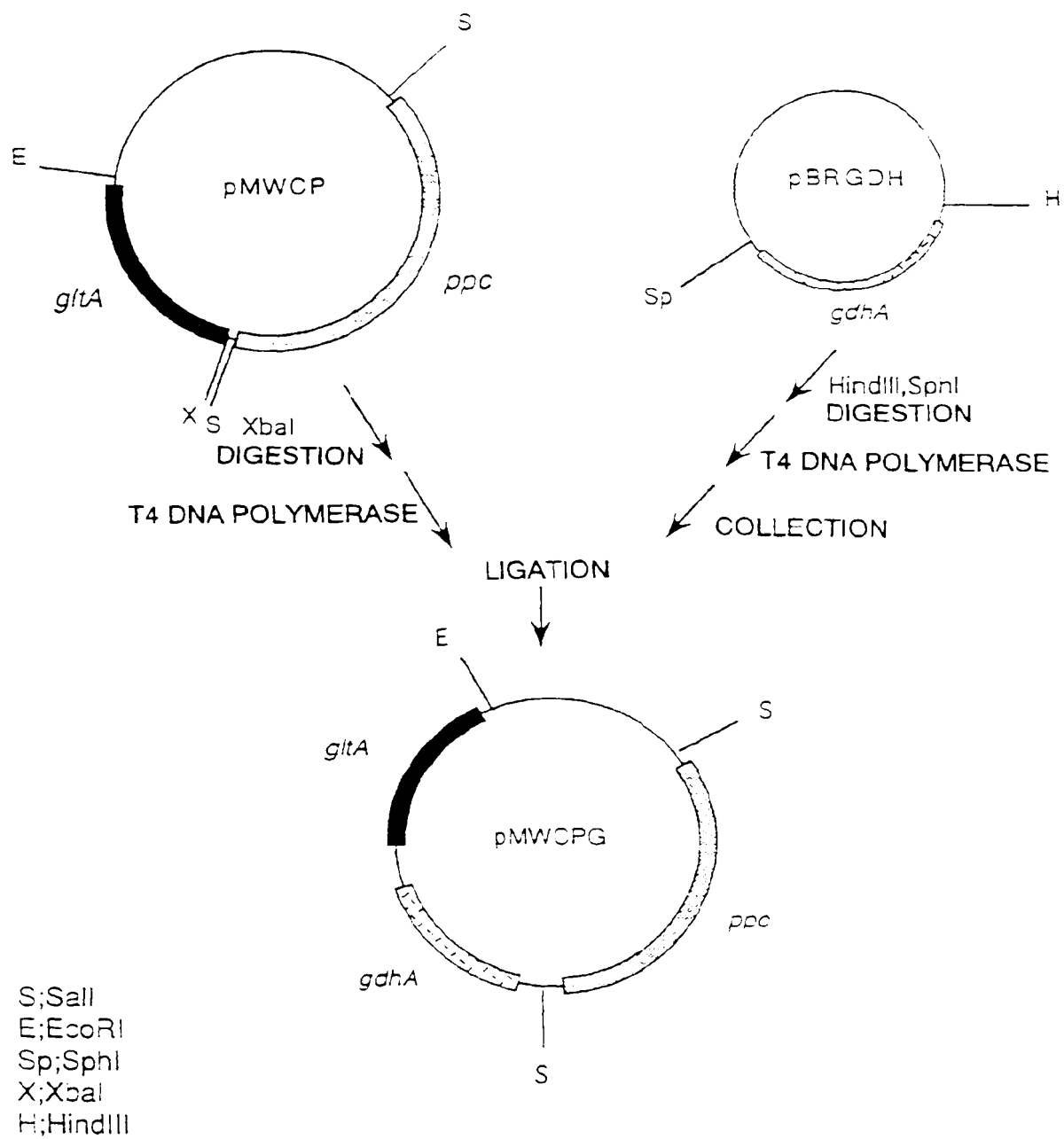


FIG. 1

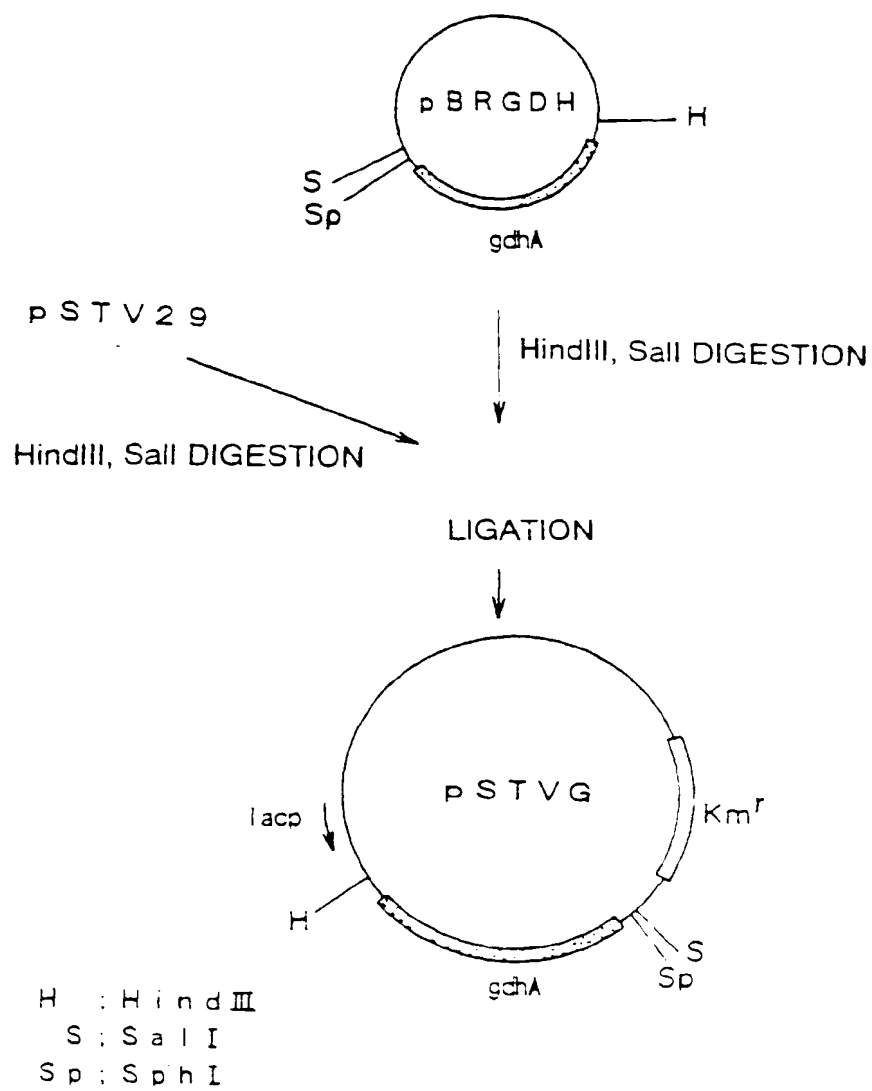


FIG. 2

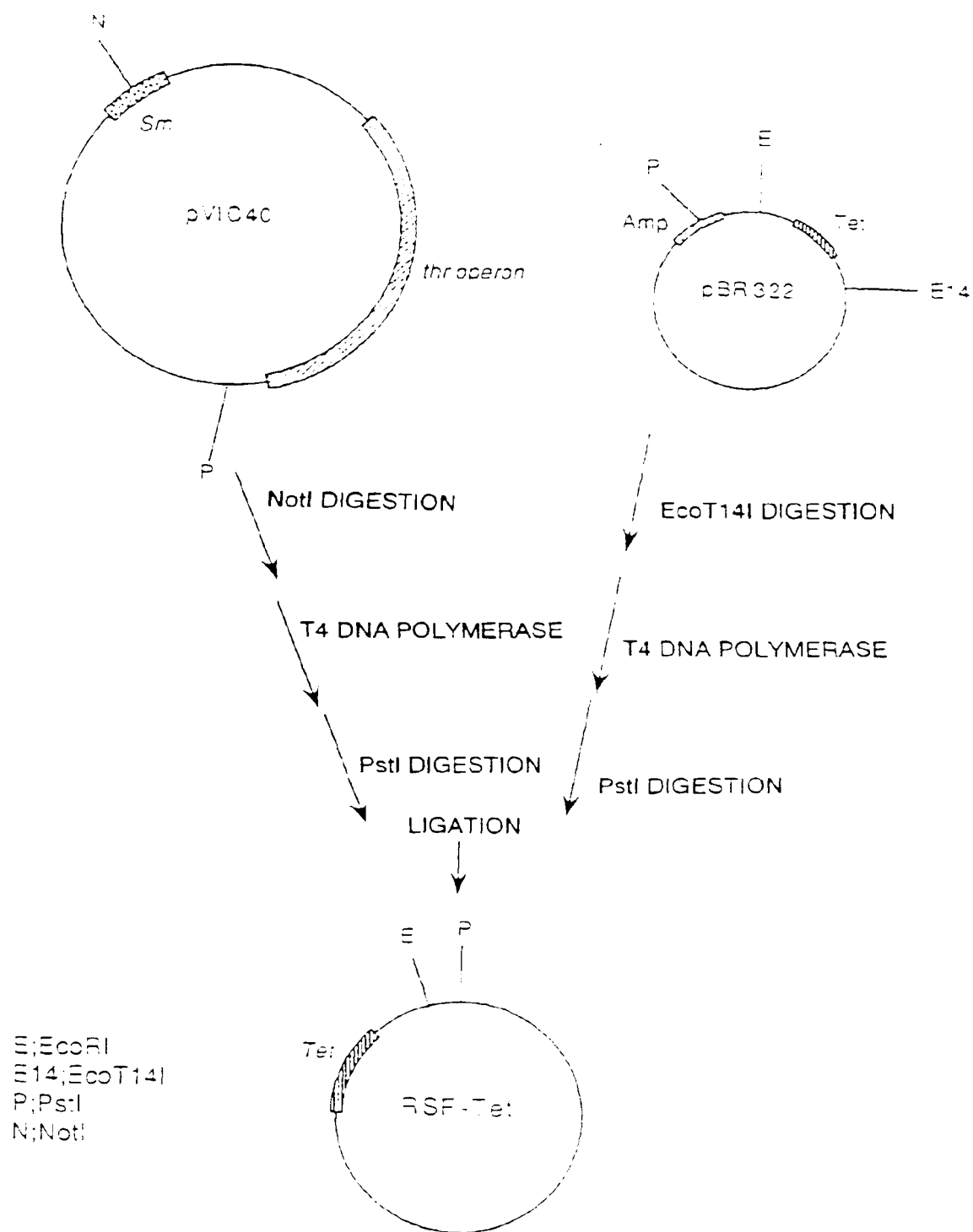


FIG. 3

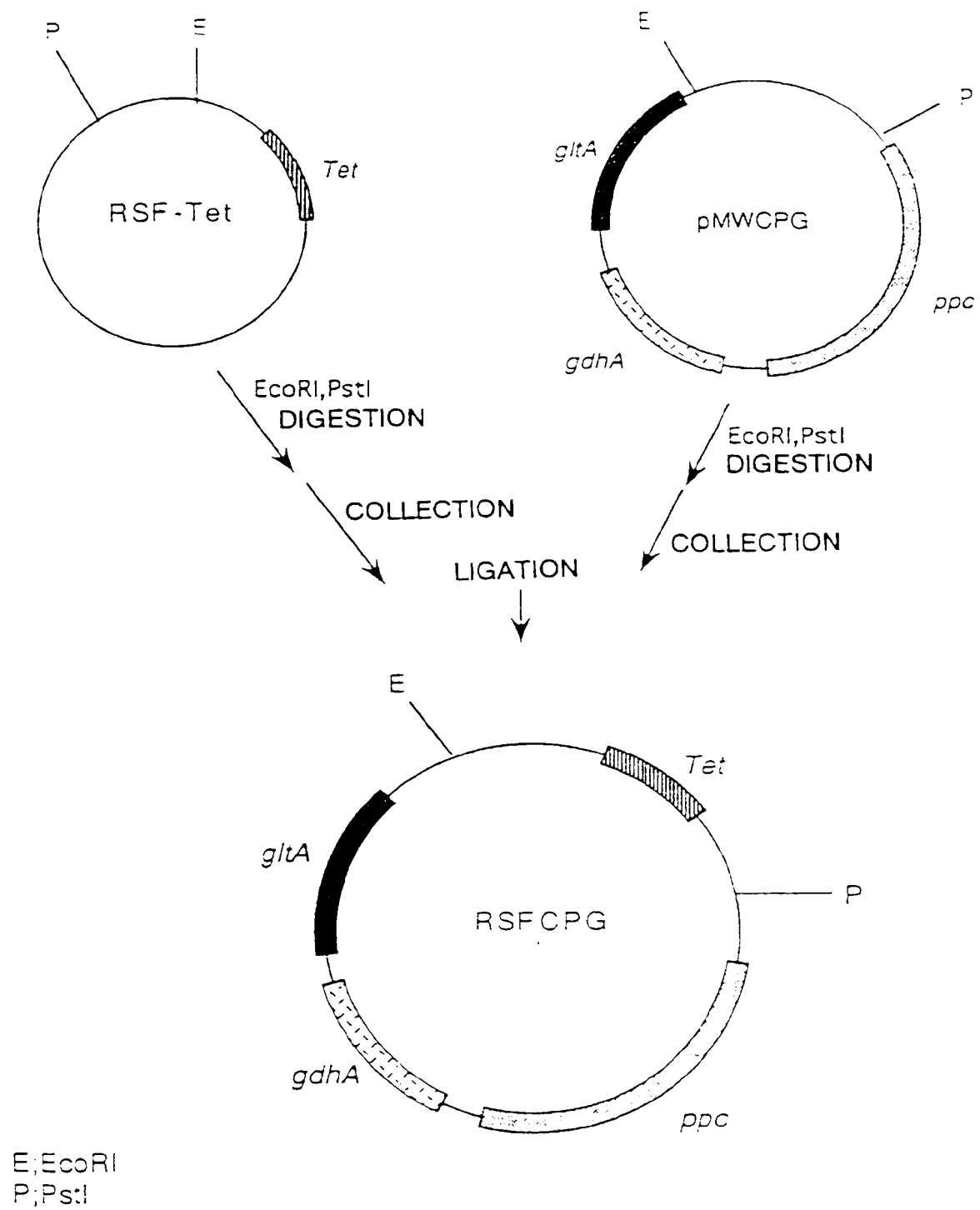


FIG. 4

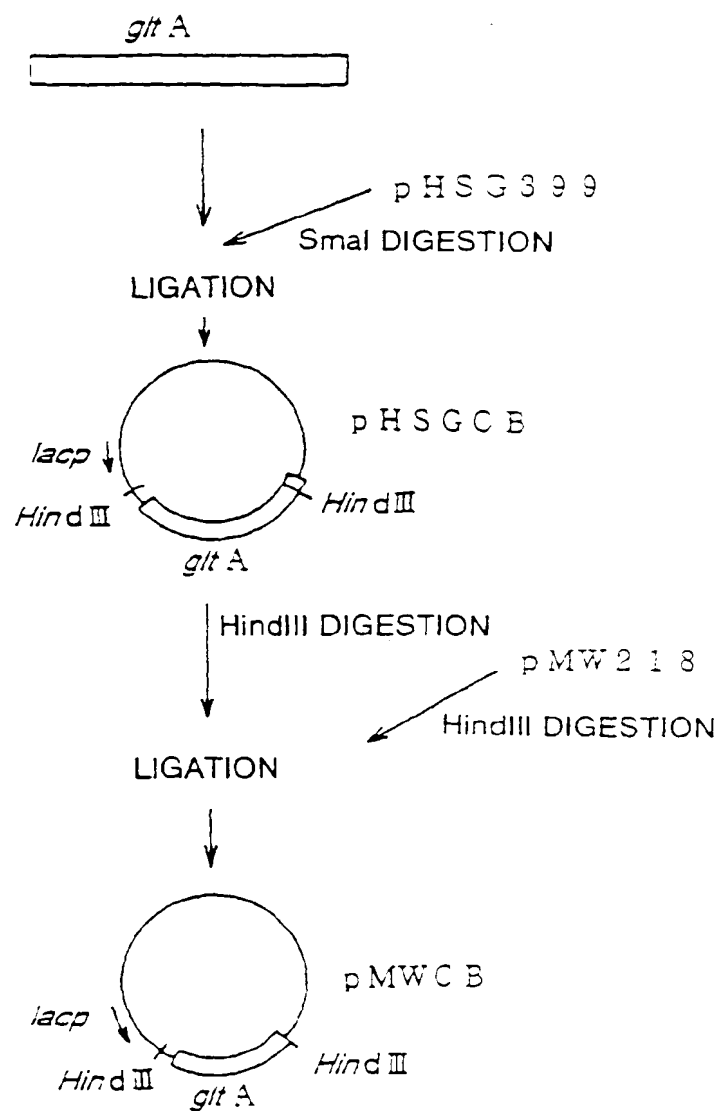


FIG. 5

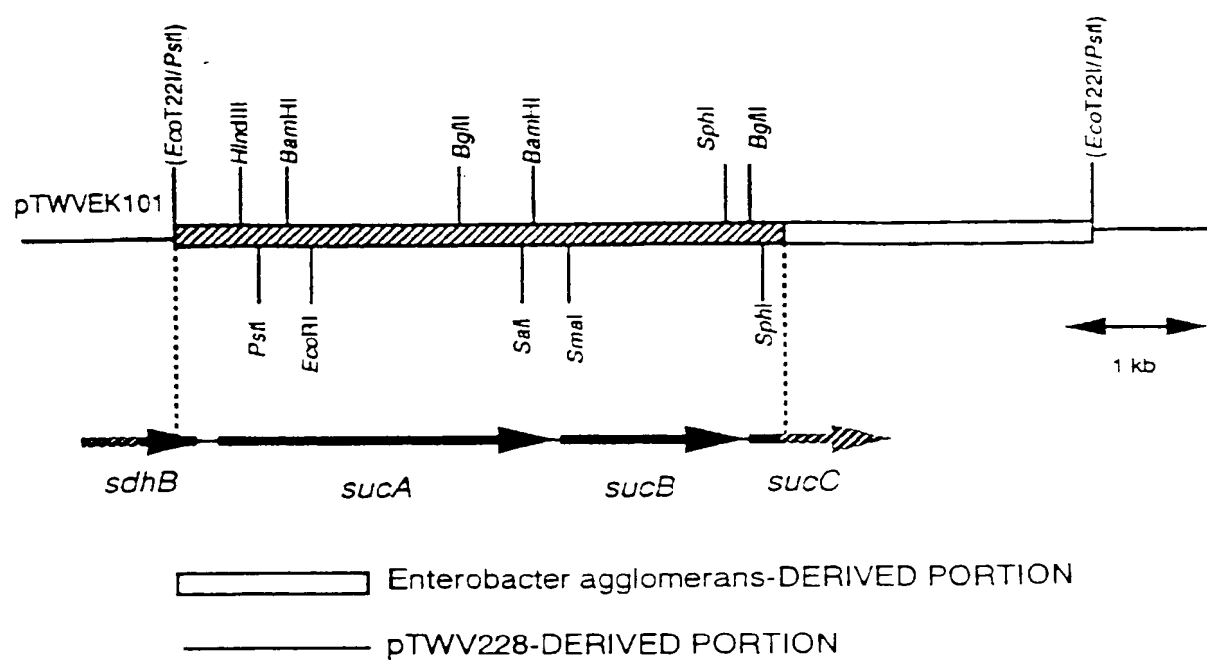


FIG. 6

[38.2% / 935 aa]

```

1'  MQNSAMKPMLDSSWLAGANQSYIEQLYEDFLTPDSVDAYNRSMFQQLPGTGVKPEQFHS
1'  MQNSALKAWLDSSYLSGANQSWIEQLYEDFLTPDSVDANWRSTFQQLPGTGVKPDQFHS
61'  ATREYFRRLAKDASRYTSSVTDPATNSKQVKVQLINAFRFRGHQEANLDPLGLWKQDRV
61'  QTREYFRRLAKDASRYSSISDPDTHVKQVKVQLINAYRFRGHQHANLDPLGLWQQQKV
121'  ADLDPAFHDLTDADFQESFNVGSFAIGKETHKLADLFDALKQTYCGSIGAEYMHINTEE
121'  ADLDPSFHDLTEADFQETFNVGSFASGKETHKLGLLEALKQTYCGPIGAEYMHITSTEE
181'  KRWIQQRIESGASQTSFSGEEKKGFLKELTAAEGLEKYLGAKFPGAKRFSLEGGDALVPM
181'  KRWIQQRIESG--RATFNSEKKRFLSELTAAEGLERYLGAKFPGAKRFSLEGGDALIPM
241'  LREMIRHAGKSGTREVVLQMAHRGRLNVLINVLGKKPQDLDFEFSGKHKEHLGTGDVKYH
239'  LKEMIRHAGNSGTREVVLQMAHRGRLNVLNVLGKKPQDLDFEFAGKHKEHLGTGDVKYH
301'  MGFSSDIETEGGLVHLALAFNPShLEIVSPVVMGsvRARLDRLAEPVSNKVLPTIHGDA
299'  MGFSSDFQTDGGLVHLALAFNPShLEIVSPVWIGsvRARLDRLEPSSNKVLPTIHGDA
361'  AVIGQGQVQETLNMSQARGYEVGGTVRIVINNQGFTTSPKPDARSTPYCTDIGKMWLAP
359'  AVTGGQVQETLNMSKARGYEVGGTVRIVINNQGFTTSPNPLDARSTPYCTDIGKMWQAP
421'  IFHVNADDPEAVAFVTRLALDYRNTFKRDVFIDLVCYRRHGHNEADEPSATQPLMYQKIK
419'  IFHVNADDPEAVAFVTRLALDFRNTFKRDVFIDLVSYRRHGHNEADEPSATQPLMYQKIK
481'  KHFTPRKIYACRLCEGEVASQEDATEMNNLYRCDALDAGECVVPEWRPMSLHSFTWSPYLN
479'  KHFTPRKIYADKLEQEKVATLEDATEMNNLYRCDALDAGDCVVAWRPMMHHSFTWSPYLN
541'  HEWDEPYPAQVDMKRLKELALRISQVPEQIEVQSRVAKIYNDRKLMAGEKAFDWGGAEN
539'  HEWDEEYPNKVEMKRLQELAKRISTVPEAVEMQSRVAKIYGDQAMAAAGEKLDWGGAEN
601'  LAYATLVDEGIPVRLSGEDSGRGTFFHRHAVVHNQANGSTYTPLHHIHNSQGEFKWDSV
599'  LAYATLVDEGIPVRLSGEDSGRGTFFHRHAVIHNQANGSTYTPLQHIHNGQGAFRWDSV
661'  LSEEAVLAFEYGYATAEPRVLTIWEAQFGDFANGAQVVIDQFISSGEQKWGRMCGLVMLL
659'  LSEEAVLAFEYGYATAEPRTLTIWEAQFGDFANGAQVVIDQFISSGEQKWGRMCGLVMLL
721'  PHGYEQGQPEHSSARLERYLQLCAEQNMQVCPSTPAQVYHMLRRQALRGMRRLVVMSP
719'  PHGYEQGQPEHSSARLERYLQLCAEQNMQVCPSTPAQVYHMLRRQALRGMRRLVVMSP
781'  KSLLRHPLAIISSLELANCSFQPAIGEIDDLDPQGVKRVVLCSGKVYYDLLEQRKDEKT
779'  KSLLRHPLAVSSLEELANCTFLPAIGEIDDLDPKGVKRVVMCSGKVYYDLLEQRKNNQH
841'  DVAIVRIEQLYPEPHQAVQCEALKAYSHVQDFVWCQEELNQGAWYCSQHHERDVPEGAT
839'  DVAIVRIEQLYPEPHKAMQEVLLQFAHYKDFVWCQEELNQGAWYCSQHHEREVPEGAS
901'  LRYAGRPASASPAVGYSVHQQQQDLYNDALNVN
899'  LRYAGRPASASPAVGYSVHQQQQDLYNDALNVE

```

FIG. 7

[88.2% / 407 aa]

```

1'  MSSVDILVPDLPESVADATVATMHHKPGDAVSRDEVIVEIETDKVVLVPASADGVLEAV
   .....
1"  MSSVDILVPDLPESVADATVATMHHKPGDAVVRDEVIVEIETDKVVLVPASADGILDAV
   .....
61'  LEDEGATVTSRQILGRLKEGNSAGKESSAKAESNOTTPAQRQTASLEEESDALSPAIRR
   .....
61"  LEDEGTTVTSRQILGRLREGNSAGKETSAKSEEKASTPAQRQQASLEEQNNDALSPAIRR
   .....
121'  LIAEHNLDAAQIKGTGVGGRLTREDVEKHLANKPQAEKAAAPAGAATAQQPVANRSEKR
   .....
121"  LIAEHNLDASAIXGTGVGGRLTREDVEKHLAKAPAKE--SAPAAAAAPAAQPALAARSEKR
   .....
181'  VPMTRLRKRVAERLLEAKNSTAMLTTFNEINMKPIMDLRKQYGDAFEKRHGVRGFMSEFY
   .....
179"  VPMTRLRKRVAERLLEAKNSTAMLTTFNEVNMKPIMDLRKQYGEAFEKRHGIRLGFMSEFY
   .....
241'  IKAVVEALKRYPEVNASIDGEDVVYHNYFDVSIKVSTPRGLVTPVLRDVALSMADIEKK
   .....
239"  VKAVVEALKRYPEVNASIDGDDVVYHNYFDVSMKVSTPRGLVTPVLRDVALSMADIEKK
   .....
301'  IKELAVKGRDGKLTVDLTGGNFTITNGGVFGSLMSTPIINPPQSAILGMHAIKDRPMAV
   .....
299"  IKELAVKGRDGKLTVEDLTGGNFTITNGGVFGSLMSTPIINPPQSAILGMHAIKDRPMAV
   .....
361'  NGQVVILPMYLAISYDHRIDGRESVGYLVAVKEMLEDPARLLLDV
   .....
359"  NGQVEILPMYLAISYDHRIDGRESVGYLVAVKEMLEDPARLLLDV

```

FIG. 8

[95.1% / 41 aa]

```

1'  MNLHEYQAKQLFARYGMPAPTGYACTTPREAEAAASKIGAG
   .....
1"  MNLHEYQAKQLFARYGLPAVGYACTTPREAEAAASKIGAGPMVVKCQVHAGGRGKAGGV

```

FIG. 9

[97.4% / 39 aa]

```

1'  AFSVFRCHSIMNCVSVCPKGLNPTRAIGHIKSMLLQRSA
   .....
181"  FLIDSRDTETDSRLDGLSDAFSVFRCHSIMNCVSVCPKGLNPTRAIGHIKSMLLQRNA

```

FIG. 10